Illuminating a bird's world

Effects of artificial light at night on avian ecology

Maaike de Jong

Thesis committee

Promotor
Prof. Dr Marcel E. Visser
Professor of Ecological genetics
Wageningen University
&
Head of department, Department of Animal Ecology
Netherlands Institute of Ecology (NIOO-KNAW), Wageningen

Co-promotor
Dr Kamiel Spoelstra
Junior group leader, Department of Animal Ecology
Netherlands Institute of Ecology (NIOO-KNAW), Wageningen

Other members
Prof. Dr Marc Naguib, Wageningen University
Dr Franz Hölker, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany
Prof. Dr Roelof A. Hut, University of Groningen
Prof. Dr Michaela Hau, Max Planck Institute for Ornithology, Seewiesen, Germany

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Chapter 1

General introduction

1.1 Disappearance of dark nights

Described by Mizon (2012) as one of the saddest paradoxes of modern life is the fact that our developing technology can provide us with stunning images of the near and far universe, and at the same time blind our eyes to the stars above. The cause of the latter is light pollution; the alteration of natural light levels in the outdoor environment due to artificial light sources (Cinzano et al. 2000). The sun, moon and stars have illuminated our lives since earliest times. Then, we learned to domesticate fire, and nowadays we fill our homes and streets with artificial lighting (Mizon 2012). Natural night-time darkness has disappeared across large parts of the world as a result of anthropogenic lighting of the environment. Thousands of stars should be visible by the human eye from a dark place, but it is difficult to find such places nowadays. Light pollution has shown a worldwide increase in the last century, especially the last six decades (Hölker et al. 2010). Nineteen percent of the Earth's surface experiences nocturnal illumination from artificial sources and one-fifth of the world's population lives in areas where the Milky Way can no longer be seen by the naked eye (Cinzano et al. 2001; Figure 1.1). Artificial lighting of urban and rural areas is predicted to continue to increase worldwide in the future.
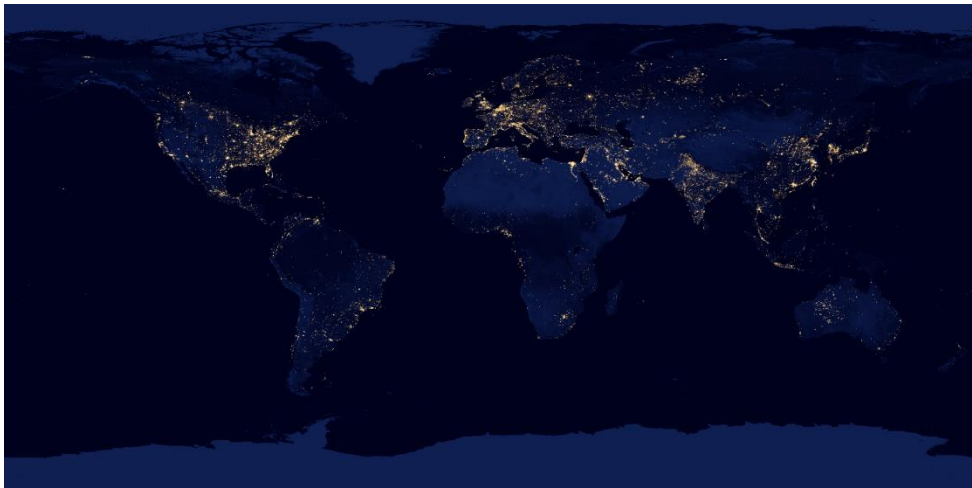
Figure 1.1 Composite image of the Earth at night. Assembled from data acquired by the Suomi National Polar-orbiting Partnership (Suomi NPP) satellite over nine days in April 2012 and 13 days in October 2012. The night-time view was made possible by the "day-night band" of the Visible Infrared Imaging Radiometer Suite (VIIRS). NASA Earth Observatory image, using Suomi NPP VIIRS data provided by NOAA National Geophysical Data Center.

Light pollution is generally being considered a problem for humans: not only is the increase of night sky brightness damaging our perception of the starry sky (McNally 1994), light pollution can also have severe impacts on human health (Cho et al. 2015). Potential long term health consequences are difficult to demonstrate experimentally in humans, but a positive association between obesity and exposure to light at night in British women was reported (McFadden et al. 2014) and a correlation with breast cancer has been found (Stevens 2009). Although the underlying mechanisms are still unclear, sleep deprivation and the suppression of melatonin production, a hormone normally produced during the night, are likely involved (Dominoni et al. 2016). The daily timing and amount of sleep has changed when humans transitioned from nomadic hunter-gatherer groups to agricultural, and later industrialized, societies (Ekirch 2006). When comparing sleep rhythm between two traditionally hunter-gatherer communities in Argentina, one with and one without 24 hour access to electricity, de la Iglesia et al. (2015) found that people in the community with electricity slept significantly shorter than those in the community without.

Not less important and increasingly the subject of studies over the past decades, artificial light at night can have strong impacts on nature (Rich and Longcore 2006). Hölker et al. (2010) stated that the loss of dark nights across the world will be a biodiversity threat, because of the major effects of light at night on behaviour and fitness of wild species. The reason why light pollution has a profound effect on wildlife may be that organisms have evolved under a natural light-dark cycle with high levels of light in daytime and very low levels of light at night. This daily and yearly cycle is the main driver for animal's circannual and circadian rhythms (Dawson et al. 2001). Light at night can change perception of day length and the natural light-dark cycle can therefore be disturbed by anthropogenic light at night, which may perturb the temporal organization of organisms. Evidence of short-term effects of nocturnal illumination on animal behaviour and physiology is accumulating (Swaddle et al. 2015). Research has only recently focused on changing light conditions at night and our understanding of the long-term ecological consequences of light pollution is still limited (Rich and Longcore 2006; Gaston et al. 2013; Gaston et al. 2015). To uncover ecosystem-wide consequences, some long-term experiments have been set up, e.g., in Germany (Hölker et al. 2015), the United Kingdom (Bennie et al. 2015) and the Netherlands (Spoelstra et al. 2015). These are providing important insights in impacts of light at night on plant and animal populations, such as the suppression of flowering (Bennie et al. 2015), the alteration of microbial communities (Hölker et al. 2015) and the suppression or facilitation of mammal activity (Spoelstra et al. 2015). The European COST Action Loss of the Night Network (LoNNe) aims to bring together actors from science, health care, public authorities and industry to

eventually influence the development path of modern lighting technology, and to create guidelines for lighting concepts that are ecologically, socially, and economically sustainable. Since its start in 2012, LoNNe has boosted the study on biological impacts of light at night by bringing together research groups from all over Europe.

Effects of artificial light at night on an individual can either be direct or indirect. For example, the fatal attraction of sea turtle nestlings to coastal lights (Kamrowski et al. 2014) is a very direct and often lethal effect. A well-known response of birds is attraction to artificial light, which causes mortality from collisions with human-made illuminated structures (Longcore et al. 2013), high mortality of seabird fledglings due to fatal collisions and higher predation (Rodríguez et al. 2014), and death of songbird nocturnal migrants due to exhaustion at light sources (Jones and Francis 2003). This immediate mortality through attraction to light has a direct impact on populations. On the other hand, more subtle effects can occur due to the disruption of natural daily cycles of light and darkness as well as seasonal cycles in day length that are used to anticipate environmental changes (Bennie et al. 2014). For example, light at night could attract prey species for an individual which consequently may change its foraging activity (like bats feeding on moths near lamps; Wakefield et al. 2015). In this case, the eventual impact may be more indirect, via effects of light at night on behaviour and physiology, which affect an individual's fitness and consequently has effects on population dynamics (Spoelstra et al. 2015). Even more indirect effects may occur by changes in the environment under influence of nocturnal illumination. Habitat suitability may be altered, and populations of prey or predator species may be affected. On an ecosystem level, this could lead to cascading effects of artificial light at night (Bennie et al. 2015). These effects may only emerge after a prolonged period of exposure to light and so far we know little about them (Gaston and Bennie 2014; Spoelstra et al. 2015).

1.2 Nocturnal illumination and birds

Birds are visual creatures with superb eyes. They have perhaps the most comprehensive visual system of all vertebrates (Goldsmith 1990). Birds occur across all regions of the world, and in all habitat types; from pristine nature to highly urbanised areas. Therefore, they are a species group potentially greatly affected by light pollution. In birds, photoperiod is an important factor determining daily patterns as well as seasonal timing. Birds align their activity and physiology to the appropriate time of the day and time of the year, via stimulation of photoreceptors by daylight to synchronize their internal circadian and circannual clocks (Dawson et al. 2001). Birds possess a wide range of photoreceptors,

located in the retina of the eye, the hypothalamus and the pineal gland. These photoreceptors all use opsin-based photo pigments to detect and transduce light (Dominoni 2015). The circadian rhythm is controlled through all three photosensitive tissues (Gwinner and Brandstätter 2001), while the hypothalamus is suggested to be involved in maintaining the circannual rhythm (Davies et al. 2012). Most birds are diurnal, but yet can still be severely affected by light at night. Artificial night lighting is hypothesized to affect the perceived photoperiod and thereby change the behaviour of birds, which in turn might affect their fitness (Farner 1964).

Indeed, artificial light at night can affect many aspects of a bird's life. Recent studies on bird populations in the wild have shown that blackbirds (*Turdus merula*) in more light polluted areas perceive a longer subjective day than conspecifics in darker regions (Dominoni and Partecke 2015) and that onset of daily activity advances in urban compared to rural sites; birds exposed to higher light intensities become active earlier in the morning (Dominoni et al. 2014). Blackbirds in urban areas also sing earlier due to anthropogenic noise and light (Nordt and Klenke 2013), and they can extend foraging activity into illuminated nights (Russ et al. 2015). In general, timing of dawn and dusk singing in common songbirds is altered by the presence of street lights (Kempenaers et al. 2010; Nordt and Klenke 2013; Da Silva et al. 2014; Da Silva et al. 2015). Great tits (*Parus major*) that were exposed to light inside their nest box woke up and left their nest box earlier in the morning (Raap et al. 2015), while great tits exposed to light just outside their nest box increased chick feeding rates (Titulaer et al. 2012). An experimental study in a wild godwit (*Limosa limosa*) population revealed that early arriving godwits chose nest sites at greater distance from road lighting than late arriving birds (de Molenaar et al. 2006). In addition, an effect of artificial light on timing of reproduction was shown in the blue tit (*Cyanistes caeruleus*; Kempenaers et al. 2010). In captivity, the effects of nocturnal illumination on avian daily rhythms have been studied in more detail. Locomotor activity was higher in blackbirds under light at night and increased sharply before dawn, when melatonin levels were decreased compared to birds under dark nights (Dominoni et al. 2013b). Behavioural effects could thus be mediated by melatonin. Measurements done in the same experimental set-up have shown that nocturnal illumination advanced the reproductive physiology of blackbirds on a short term basis (Dominoni et al. 2013a), but suppressed reproductive activity in the long run (Dominoni et al. 2013d). This still relatively small set of studies all demonstrate rather short term effects of light pollution on the behaviour of birds. Experimental studies on the effect of light at night on behaviour and fitness in a field situation with no other anthropogenic disturbance are so far lacking (Spoelstra and Visser 2014).

Unravelling the mechanisms underlying the effects of artificial light on individuals is among the main goals in the field of light pollution ecology research. To understand which mechanisms may be affected by light at night the focus needs to be on light as a physiological signal, and on how photic information is perceived, decoded and transmitted (Dominoni 2015). The hormone melatonin plays an important role in the circadian organisation of birds and other vertebrates and is released by the pineal gland during the dark phase of the day and suppressed by light via photoreceptors (Bell-Pedersen et al. 2005; Cassone 2014). It encodes the duration of the night and thus day length, thereby helping birds to synchronise their behaviour and physiology to the external light-dark cycle (Gwinner et al. 1997). Melatonin is known to be related to locomotor activity and both are regularly measured in relation to effects of light at night (Dominoni et al. 2013b; Yadav et al. 2015). Suppression of melatonin levels by light at night was recently shown in the tammar wallaby (*Macropus eugenii*; Robert et al. 2015), the Indian weaver bird (*Ploceus philippinus*; Singh et al. 2012) and in the blackbird (Dominoni et al. 2013b). In fish, circadian melatonin patterns were inhibited by low intensity night light levels (Brüning et al. 2015).

In the end, the most relevant effects of artificial light at night are those on the population level. All demographic parameters, immigration, births, deaths, and emigration, of local populations need to be studied in order to ultimately assess spatial patterns of species richness and composition in a certain area (Gaston and Bennie 2014). Long-term studies that attempt this are, so far, scarce. A four-year study at Réunion Island shows that light-induced mortality of petrel fledglings is annually high, but also shows that disturbance of the population dynamics of this long lived seabird has not yet been detected (Le Corre et al. 2002). Data on numbers of observations of different bird species from a large-scale experiment show that, during the first two years, densities of birds seem to increase in illuminated compared to dark areas (Spoelstra et al. 2015), but monitoring needs to be continued to identify longer term consequences on population level. The mechanisms of response to anthropogenic light described so far involve immediate adjustments to, for example, behaviour or physiology. Another mechanism could operate through alterations of developmental processes and gene expression which are influenced by environmental conditions as a bird develops its phenotype (Swaddle et al. 2015). Evolutionary changes could occur in response to light pollution because of the extent to which it forms novel, previously unknown, environmental conditions.

1.3 Reducing impact of light pollution

The research interest in the biological impacts of artificial light at night has grown tremendously over the last years (Gaston et al. 2015), and the interest in mitigation measures has become evident. There are many options to reduce the effects of night-time light pollution on ecosystems, the five main ones have been discussed by Gaston et al. (2012). Simply illuminating the environment less would also save a lot of energy; light pollution might actually cost less to solve than it does to continue as it is. Keeping natural, unlit areas dark (1) may therefore seem the simplest option. As an example, the International Dark-Sky Association started the Dark Sky Places Program in 2001 to encourage communities around the world to preserve and protect dark sites through responsible lighting polices and public education. Nowadays, there are over 50 Dark Sky Places, mainly across the Western world. No light at night might however not always be possible or even allowed. Changing the duration of lighting (2), for example by only illuminating during certain hours of the night, or reducing trespass of lighting (3), in other words, spill of light into the sky, by changing lamp design are both plausible approaches to reduce impacts of light pollution. One of the important research challenges is to determine the thresholds and dose-response functions for biological impacts of artificial light at night (Gaston et al. 2015). Most studies so far have focused on light at night versus no light at night, whereas the presence of light at night cannot be indicated as a 'yes or no' event, but is a disturbance of natural habitat which continues from bright light close to the light source to very low light intensities at greater distance. Knowing the behavioural response of organisms to different light intensities could help to advice on dimming of lamps, and by changing the intensity of lighting (4) reducing effects on wildlife. Finally, adaption of the light spectrum (5) would be another option.

Modern light-emitting diode (LED) lamps are used more and more in outdoor lighting, mainly because of their economic advantages (Tan et al. 2012). Another important advantage of LED lamps is that their colour composition can be custom designed. With different organisms being sensitive in different parts of the light spectrum, the responses of organisms to artificial light at night could be highly dependent on the spectral composition of the light (Perkin et al. 2011). This, combined with the worldwide change to LED lighting, makes it relevant to study and understand the organismal responses to differences in the spectral composition of light sources (Spoelstra et al. 2015). Ultimately, it should be possible to advise on the use of lamps with certain characteristics (such as spectral composition and light intensity) for a specific setting in such a way that the emitted light has the sufficient quality to support human activities and, at the same time, has minimal effects on biological processes of the organisms or

communities nearby (Musters et al. 2009). To enable necessary, full colour vision for humans, light needs a continuous and broad spectrum, which can be adapted by amplification or reduction of spectral power at parts of the spectrum (Figure 1.2). Two suitable spectral compositions have been created and are commercially available; green light, with increased blue and reduced red emission, and red light, with increased red and reduced blue emission. Effects of these light colours, in comparison to 'traditional' white LED light, need further investigation to see whether there is potential for mitigation of impact of light on flora and fauna via adaptation of the light spectrum (Spoelstra et al. 2015). Outdoor lighting light levels are standardized in lux, a measure of illumination based on human vision, where lamps of different colours with the same intensity in lux are perceived by humans as equally intense. An additional complication is that lamps with different spectra might not only differ in colour, but also in intensity for organisms other than humans, because of differences in spectral sensitivity.

Birds are sensitive to a range of wavelengths to which humans are not (Bennett and Théry 2007). Many birds have ultraviolet (UV) vision, and most birds perceive colours through four cone types (Bennett and Cuthill 1994; Osorio and Vorobyev 2008). Besides, as discussed above, birds have extra-ocular light perception by photoreceptors in the pineal gland and brain (Cassone 2014). The spectral absorption characteristics of the photoreceptors in the eyes of several passerines have been measured (Bowmaker et al. 1997; Hart et al. 2000). The role of spectral composition in the impact of nocturnal illumination on avian behaviour and fitness has so far been poorly studied (Musters et al. 2009). The effects of artificial light at night of different spectral composition on avian ecology are difficult to predict, because of the excellent, but complicated visual system of birds (Spoelstra and Visser 2014). Besides, knowing the absorption spectrum of a species does not necessarily directly translate into knowing the action spectrum; the effect of the relative abundance of light of specific wavelengths on behaviour is yet another question. Apart from the effects of perceived intensity of different coloured light by birds, there may also be a direct effect of the spectrum, but few things are known about effects of light with different colours. Long wavelengths are able to penetrate the skull more easily than short wavelengths, and are more effective at inducing a photoperiodic response (Hartwig and van Veen 1979), stimulating gonadal growth (a measure of reproductive readiness; Kumar et al. 2000b) and promoting body fattening than shorter wavelengths (Malik et al. 2002). Blackheaded buntings (*Emberiza melanocephala*) and Indian weaverbirds interpreted short (blue) and long (red) light wavelengths applied at equal energy levels as the day and night, respectively, indicating that they perceived blue light as being more intense (Yadav et al. 2015). Nocturnally migrating birds seem to be disoriented by, and attracted to, white and red, but less to green and blue light

(Wiltschko and Wiltschko 1995; Poot et al. 2008), but see also Evans (2010) for some critical thoughts. The removal of red light from the spectrum makes this effect less pronounced (Wiltschko et al. 1993; Poot et al. 2008). A possible mechanism is that cryptochrome receptor molecules are dependent on short-wavelength light, which aligns with the wavelength dependency of magnetoreception observed in behavioural tests (the avian radical pair mechanism hypothesis; Liedvogel et al. 2007; Rodgers and Hore 2009). All in all, results so far are not very consistent and it is not yet clear how light with different spectral composition affects the behaviour and fitness of birds.
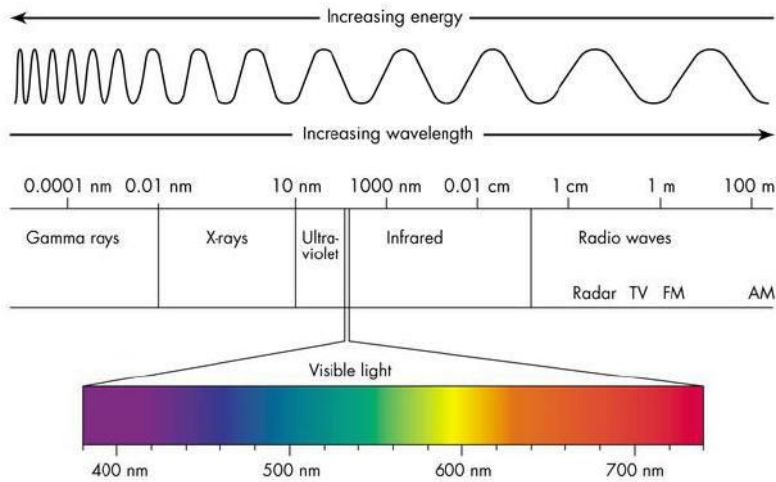
Figure 1.2 Overview of the range of wavelengths, with close-up on the, for humans, visible part of the spectrum (visible light).

1.4 Aim and outline of thesis

The worldwide disappearance of dark nights due to light pollution, its potential large impact on ecosystems of which birds often form an important component, and the possibilities of reducing this impact by adapting management strategies, constitute the foundation for the work in this thesis. The overall aim of this thesis was to investigate the effects of artificial light at night on the ecology of birds. More specifically, this was done by studying the effects of different light colours and light intensities on several aspects of ecology, amongst which physiology, behaviour, life-history traits and fitness, of common songbirds in the Netherlands (Figure 1.3).

To study the ecology of birds in illuminated nights, I used three different approaches. The first approach is using long term data from across the

Netherlands, in order to look at levels of light pollution in relation to avian timing of breeding. In this correlational study (**Chapter 2**), I used satellite data of night-time light levels as a proxy for urbanization and linked these to first egg laying dates of the ten most common nest box breeding birds, gathered by citizen scientists throughout the Netherlands. Timing of breeding is an important life-history trait which is expected to be affected by light at night, as well as by other variables closely linked to urbanization.
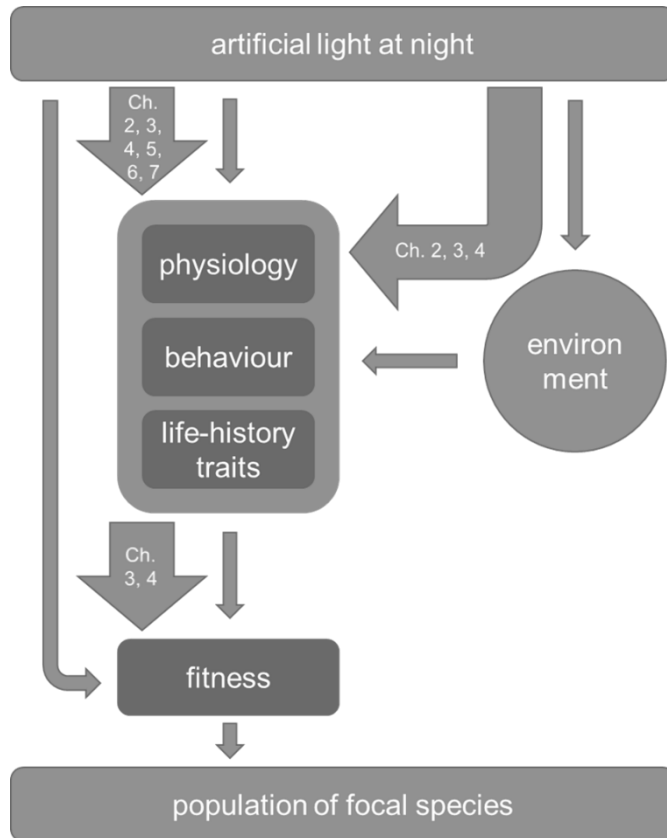
Figure 1.3 Flowchart on how artificial light at night can affect an organism at different organisational levels. Indicated with the orange arrows on which direct and / or indirect effects of light at night I focus in each chapter of this thesis. Adapted from Spoelstra et al. (2015), with permission.

The second approach was to perform a large-scale field experiment. This thesis is part of the Light on Nature project, a cooperation between scientists of the Netherlands Institute of Ecology (NIOO-KNAW) and Wageningen University and

Research Centre (WUR), which started in 2011 and is still ongoing. The goal of the Light on Nature project is to assess the long-term effects and mechanisms behind these effects of street lighting on flora and fauna, based on a large-scale, experimental set-up. Eight study sites in the Netherlands have been set up where natural, formerly dark, habitat is experimentally illuminated (Spoelstra et al. 2015). Effects of exposure to three different light spectra (white, green and red light), compared to a dark control, on different species groups are being monitored according to standardized protocols, in close cooperation with the following Dutch NGOs; Dutch Butterfly Conservation (Vlinderstichting), Dutch Centre for Field Ornithology (Sovon), Dutch Mammal Society (Zoogdiervereniging), Dutch Centre for Avian Migration and Demography (Vogeltrekstation), Reptile, Amphibian & Fish Conservation Netherlands (RAVON) and Dutch Foundation for Botanical Research (FLORON). The studies in **Chapters 3**, **4** and **5** of this thesis have been performed at the Light on Nature study sites.

At each of these sites, rows with lamp posts have been placed perpendicular to the forest edge, from inside the forest, into the adjacent open field. Bird nest boxes have been placed in the forest, following a standardized pattern around the lamp posts, in order to study the breeding ecology of cavity-breeding passerines. By doing so, the effects of light at night on individuals nesting at different distances from the lamps can be measured. The experimental nature of this set-up allows for testing the effects of nocturnal illumination independent of other anthropogenic disturbances normally associated with light at night. In two consecutive years, I measured several life-history traits and fitness components of great tits and pied flycatchers (*Ficedula hypoleuca*), to assess the effects of light at night with different spectral composition on their breeding biology, and ultimately, fitness (**Chapter 3**). In the same two years, I investigated the effects of experimental light at night on extra-pair behaviour of great tits, by determining the parentage of all great tit offspring. Great tits are socially monogamous, but extra-pair paternity is a common phenomenon, as in many other bird species. Via effects of nocturnal illumination on dawn song, onset of activity or mate guarding behaviour, extra-pair paternity is hypothesized to be affected, and natural patterns of sexual selection processes might be disrupted (**Chapter 4**). The light levels around the lamps at our experimental study sites have been measured, but birds are highly mobile species. Therefore, it is not known to how much light at night the studied individuals are actually exposed, as they are well able to move away from the light. By deploying great tits with low intensity light loggers, I assessed what light levels they were exposed to (**Chapter 5**), to be able to eventually get an idea about whether observed effects of light at night in the field, are direct or rather indirect effects.

Although ecological field experiments may in the end be the best way to study effects of artificial light on wild birds, it is usually impossible to control or measure all variables that play a role. My third approach, in **Chapters 6** and **7**, was therefore to study the effects of night-time light on the physiology and behaviour of captive birds, in a controlled environment. I determined the dose-response relationship for the effect of night light intensity on the daily rhythms of great tits (**Chapter 6**). In a laboratory set-up, where the only difference between individuals was the intensity of night light, I measured daily activity patterns and melatonin levels. Exposing birds to different intensities of illumination might give insight in the possibilities of reducing impact of light at night by adapting light intensity. Using the same laboratory set-up, I studied the effects of light colour and light intensity on daily rhythms in blue tits (**Chapter 7**). Using lamps with the same spectral composition as those in the field experiment, suitable for application in outdoor lighting, makes that the results of this study can be directly translated into management practices.

Finally, in **Chapter 8**, I discuss how these three approaches and different studies together give insight in, and improve our understanding of, the effects of artificial light at night on the ecology of birds. Also, I discuss possible implications of our findings for lighting policy and nature conservation. Moreover, I discuss what we still do not know and how these gaps in research may be filled.

Chapter 2

Timing of avian breeding in an urbanized world

Maaike de Jong
Laura van den Eertwegh
Ronald E. Beskers
Peter P. de Vries
Kamiel Spoelstra
Marcel E. Visser

Abstract

A large part of the world is urbanized, and the process of urbanization is ongoing. Species differ in the extent to which they are impacted by urbanization, depending on adaption capacity, and on the fitness consequences when adaptation lags behind. One prominent effect of urbanization is the dramatic change of the night-time environment: in urban areas nights are no longer dark. Here, we studied the impact of urbanization on the timing of breeding, which is a key life-history trait. We used six years of data from ten common bird species, breeding in nest boxes throughout the Netherlands. We took the intensity of artificial light in the form of zenithal sky brightness and light emission, as a proxy for urbanization. We found a correlation between light levels and seasonal timing in three of the ten species (great tit, blue tit and pied flycatcher), but these relationships differed between years. The effect of urbanization on seasonal timing is at best weak in our study which was however mainly based on areas with relatively low light levels. There is a clear lack of data for breeding birds in more urbanized environments, an ever expanding habitat for an increasing number of species worldwide.

2.1 Introduction

In 2014, 54% of the world's population lived in urban areas, which is predicted to increase to 66% by 2050. In the Netherlands, ranking in the top 20 of most urban countries, 90% of all inhabitants were living in urban areas in 2014 and the annual increase in the proportion of inhabitants living in cities was among the highest in Europe (United Nations 2014). The consequences of urbanization for biodiversity conservation are largely unknown, but it has been shown that global growth of urban areas increases the probability of vertebrate species being listed as threatened (Mcdonald et al. 2008).

Implications of urbanization for nature depend on how well species can adapt. For example, generalist bird species commonly do better and are more often found in urban areas than specialists (Evans et al. 2011), although even these generalists may be affected by the urban habitat. In a meta-analysis (Chamberlain et al. 2009), overall egg laying dates were found to be advanced and clutch sizes were smaller in urban landscapes compared to non-urban. Also, nestling weights and fledging success in urban areas were lower, but on the other hand, annual productivity was usually higher in urban habitats (Chamberlain et al. 2009). Urban blackbirds (*Turdus merula*) developed their gonads approximately three weeks earlier than forest conspecifics (Partecke et al. 2005), and these differences in reproductive timing were shown to be mainly a result of phenotypic flexibility (Partecke et al. 2004). The earlier onset of breeding in an urban versus a rural population of blackbirds was also shown experimentally (Dominoni et al. 2013a).

Possible environmental parameters in the urban environment which may explain the advance in reproductive timing could be increased temperature, or food availability. For example, a study on suburban scrub-jays (*Aphelocoma coerulescens*) suggests that high-quality human-provided foods can be used to breed earlier (Schoech and Bowman 2003). However, a potentially important effect of urbanization on the environment is the increase in nocturnal light levels, and this effect is increasingly receiving attention in ecological research over the last years (Kempenaers et al. 2010; Dominoni et al. 2015; Spoelstra et al. 2015).

In urban areas, nights are no longer dark as they used to be in former times. Animal behaviour has evolved to be synchronised with the natural light-dark cycle; the received photoperiod drives yearly and daily rhythms (Dawson et al. 2001). Light at night could alter the perception of photoperiod and thereby advance seasonal timing of birds (Farner 1964). Advancement of egg laying in illuminated areas is for example shown in blue tits (*Cyanistes caeruleus*; Kempenaers et al. 2010) and in great tits (*Parus major*; de Jong et al. 2015).

Satellite data of nocturnal light form a reliable and accurate measure for urbanization level (Sutton et al. 2001; Gao et al. 2015), and night-time lights in well developed countries are positively correlated with population density and road density (Levin and Duke 2012). Therefore, we use night-time light levels as a proxy for urbanization and aim to quantify the effect of urbanization on timing of avian breeding in the Netherlands. We use two available datasets with measures of light at night; light emission and sky brightness. We hypothesize that birds will be laying their eggs on average earlier in more urbanized areas.

2.2 Methods

In this study, we make use of data on nest box breeding birds collected by NESTKAST, a Dutch citizen science network. We used in total 2148 average first egg laying dates, collected by regular checking of the nest boxes, and the numbers of recorded clutches for the ten most common species in the dataset. Study species are, in decreasing order of sample size (Table 2.1); great tit, blue tit, nuthatch (*Sitta europaea*), pied flycatcher (*Ficedula hypoleuca*), starling (*Sturnus vulgaris*), redstart (*Phoenicurus phoenicurus*), tawny owl (*Strix aluco*), tree sparrow (*Passer montanus*), coal tit (*Periparus ater*) and stock dove (*Columba oenas*). Data originates from 259 study areas in the Netherlands ranging from natural to peri-urban habitat and were collected in the years 2009-2014 (Figure 2.1).

From 2011 onwards, zenithal sky brightness (measured as luminance, from the ground), is continuously recorded at nine locations in the Netherlands. These measurements were used to validate the IPOLicht model (RIVM 2014a), with which a zenithal sky brightness map for the whole country, for nights without clouds, was calculated (RIVM 2014b; Figure 2.1A). The NOAA (USA National Oceanic and Atmospheric Administration) used records from the Visible Infrared Imaging Radiometer Suite (VIIRS), which is on board of the Suomi National Polar-orbiting Partnership satellite, to create a worldwide map of night-time light emission in 2013 (measured as radiance, from above). Records from April 18-26 and October 11-23 2012, from nights with no clouds or moonlight, were used. A selection for the Netherlands has been made available by RIVM (RIVM 2013; Figure 2.1B). Both maps are also available at Atlasleefomgeving (Atlasleefomgeving 2015). We used both measures because zenithal sky brightness is a light value at a certain point on the ground and therefore more relevant for birds, but these data are modelled rather than directly measured. Light emission data on the other hand are real measurements for each point, but viewed from a point in the sky and therefore maybe less relevant for a bird. As both these measurements are informative, despite their correlation ($r = 0.85$), we

used them both in the analysis. The sky brightness map contains values for each grid of 250 x 250 m and the light emission map for grids of 300 x 300 m. Sky brightness and light emission were calculated for each of the 259 study areas, by taking the average value of all grids within a circle of 950 m radius around the area, based on the average surface of our study areas, using ESRI ArcMap 10.1 (Esri 2012).

For each of the ten species, we ran linear-mixed-effects models (LMMs) and linear models (LMs) to investigate the relation between light value (either log of sky brightness or log of light emission) with average first egg laying dates. Lay date is weighted by the square root of the number of clutches in the area on which the average lay date is based. First, we checked whether the relation between light value and lay date differed per year (overall analysis; LMMs). If the interaction between light and year was not significant, we tested the main effect of the light value. If the relation between light value and lay date differed per year, we continued testing this relationship in the six years separately (within year analysis; LMs). In all models, latitude and longitude of the study area were provided as covariates, to account for location related differences, and in the models with all years included, study area was entered as a random effect to correct for multiple measures from the same area (in different years). Our data met the criteria for using models with normal error distributions. All statistical analyses were done using R v. 3.1.2 (R Development Core Team 2014).

2.3 Results

For three species we found a relationship between light levels and seasonal timing, which differed between years (Table 2.1). For the blue tit the average lay date was significantly negatively correlated with light value in 2012, and for the pied flycatcher this correlation was present in 2010. No correlation was present in other years for the blue tit, and the pied flycatcher showed a positive correlation in 2014. For the great tit, the correlation was not significant in any of the years (Figure 2.2). Stock doves seem to breed earlier in all years in areas with higher sky brightness and light emission, but this effect is not significant (Table 2.1 and Appendix Figure 2.A1). We found no relation of light with average first egg laying dates of the nuthatch, starling, redstart, tawny owl, tree sparrow or coal tit (in none of the years, Table 2.1 and Appendix Figure 2.A1).

2.4 Discussion

Although our analyses did not reveal a consistent effect of nocturnal light level on
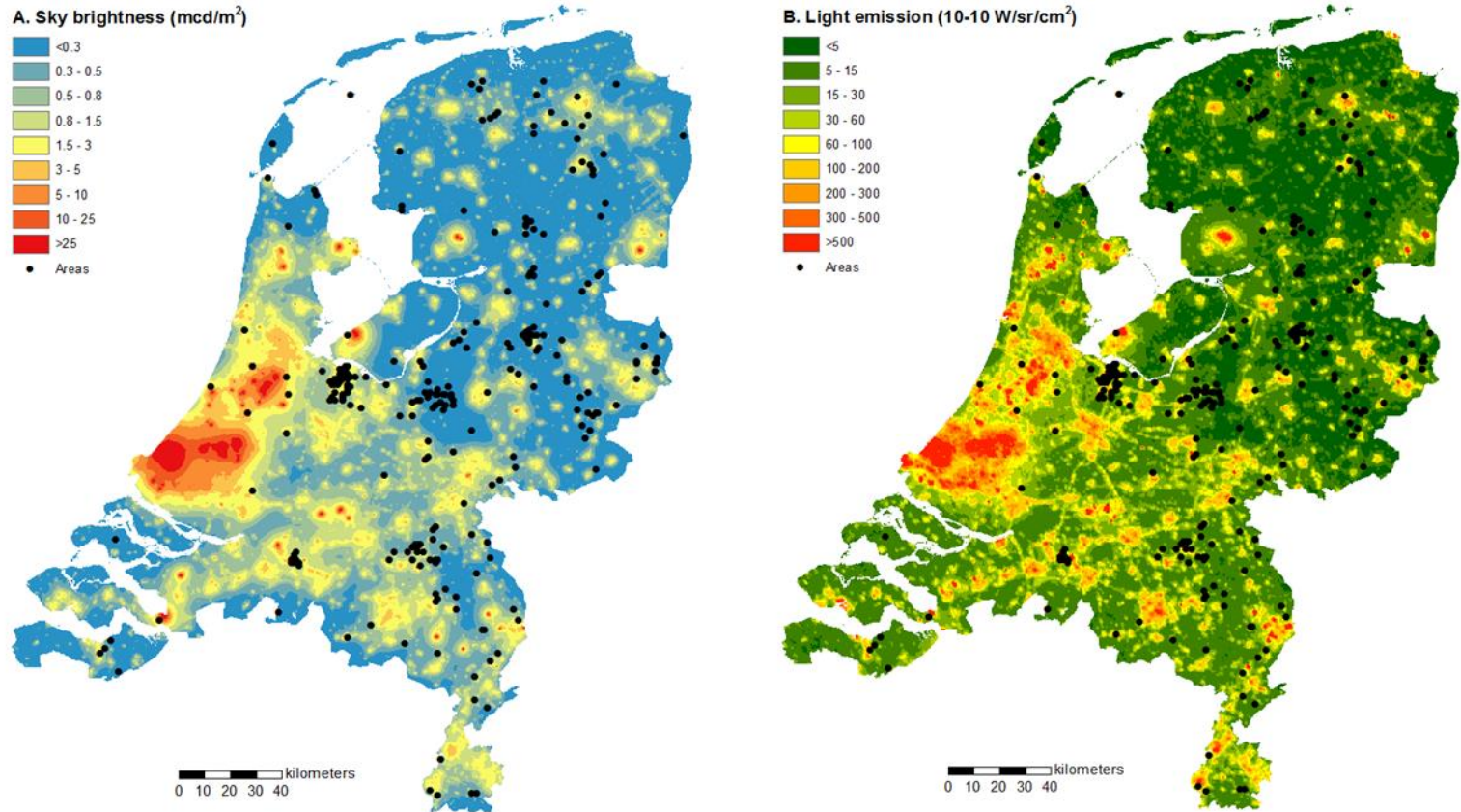
Figure 2.1 Zenithal sky brightness data (as luminance in mcd/m^2) with lay date areas (250 x 250 m grid, A). Light emission data (as radiance in 10^{-10} W/sr/cm^2) with lay date areas (300 x 300 m grid, B).
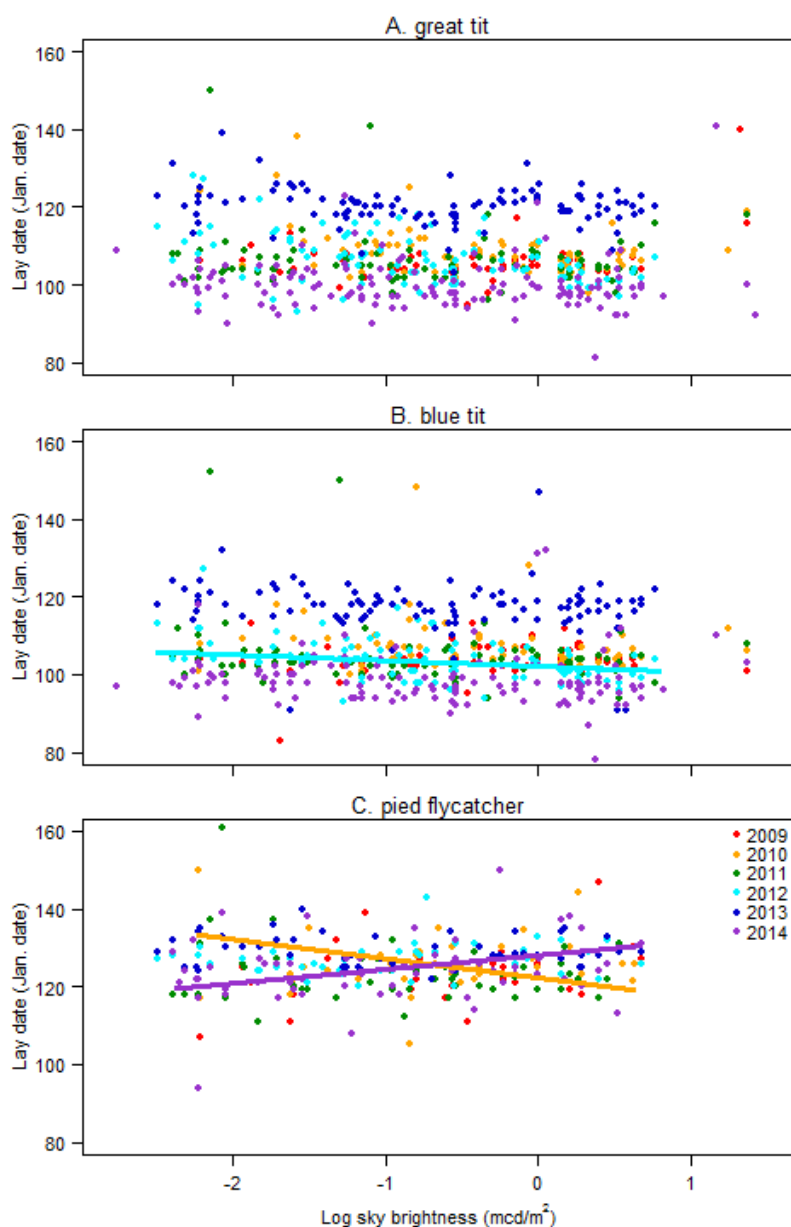
Figure 2.2 Lay date versus log of zenithal sky brightness for species for which the interaction between light and year is significant (great tit (A), blue tit (B) and pied flycatcher (C)). Each dot indicates an average first egg laying date for a specific area in a specific year. Lines are plotted for the years that light had a significant effect.

Table 2.1 Results of the linear-mixed-effects models (LMMs; overall analysis) and the linear models (LMs; within year analysis) on the relation between weighted average first egg laying dates and the interaction between light and year (overall) or light, log of zenithal sky brightness and log of light emission, (within year). Sample sizes (n), estimates and the significance level (*p*) are given for each term, *p*-values are in italics when considered significant. In the overall analysis *p* values were considered significant when <0.0025 (Bonferroni corrected for testing ten species and two light values).

		Overall analysis				Within year analysis								
						2009			2010			2011		
			Light : Year	Light			Light			Light			Light	
		n	p value	estimate	p value	n	estimate	p value	n	estimate	p value	n	estimate	p value
Great tit	Sky brightness	586	*0.0002*			75	1.11	0.31	81	-0.56	0.47	90	-0.46	0.61
Parus major	Light emission		0.005				1.67	0.05		-0.07	0.91		0.38	0.66
Blue tit	Sky brightness	549	0.009			69	0.55	0.50	74	-0.68	0.57	88	-1.44	0.09
Cyanistes caeruleus	Light emission		*0.002*				-0.06	0.93		-1.01	0.30		-0.87	0.27
Nuthatch	Sky brightness	316	0.22	-1.2	0.01									
Sitta europaea	Light emission		0.09	-0.65	0.15									
Pied flycatcher	Sky brightness	298	0.009			33	2.01	0.21	49	-4.96	0.0006	54	-1.31	0.27
Ficedula hypoleuca	Light emission		*0.001*				1.04	0.52		-4.2	*0.002*		-2.81	0.02
Starling	Sky brightness	106	0.94	0.52	0.72									
Sturnus vulgaris	Light emission		0.80	-0.45	0.77									
Redstart	Sky brightness	68	0.34	-3.17	0.25									
Phoenicurus phoenicurus	Light emission		0.25	-0.4	0.85									
Tawny owl	Sky brightness	65	0.95	1.85	0.69									
Strix aluco	Light emission		0.82	-0.07	0.98									
Tree sparrow	Sky brightness	60	0.56	-2.76	0.36									
Passer montanus	Light emission		0.54	-1.4	0.49									
Coal tit	Sky brightness	59	0.34	1.3	0.63									
Periparus ater	Light emission		0.73	5.76	0.04									
Stock dove	Sky brightness	41	0.87	-22.87	0.009									
Columba oenas	Light emission		0.73	-22.64	0.006									

Table 2.1 (*Continued.*)

		Within year analysis								
		2012			**2013**			**2014**		
			Light			Light			Light	
		n	estimate	p value	n	estimate	p value	n	estimate	p value
Great tit	Sky brightness	99	-1.06	0.15	104	-0.62	0.27	137	-0.75	0.18
Parus major	Light emission		-0.88	0.23		-0.38	0.45		-0.8	0.12
Blue tit	Sky brightness	94	-1.54	*0.03*	99	-0.87	0.33	125	-1.01	0.08
Cyanistes caeruleus	Light emission		-1.53	*0.04*		-0.77	0.31		-0.33	0.53
Nuthatch	Sky brightness									
Sitta europaea	Light emission									
Pied flycatcher	Sky brightness	50	0.9	0.29	52	-0.44	0.47	60	3.62	*0.005*
Ficedula hypoleuca	Light emission		0.45	0.57		-0.45	0.43		1.56	*0.0003*
Starling	Sky brightness									
Sturnus vulgaris	Light emission									
Redstart	Sky brightness									
Phoenicurus phoenicurus	Light emission									
Tawny owl	Sky brightness									
Strix aluco	Light emission									
Tree sparrow	Sky brightness									
Passer montanus	Light emission									
Coal tit	Sky brightness									
Periparus ater	Light emission									
Stock dove	Sky brightness									
Columba oenas	Light emission									

average first egg laying dates, we did observe a correlation between lay dates and light levels, our proxy for the degree of urbanization, for species with large sample sizes. Great and blue tits are common breeders in cities and the presence of a correlation between seasonal timing and urbanization may originate from adaptation to urban habitats (Swaddle et al. 2015), whereas other species may have only recently colonized cities and therefore a correlation may not be present yet.

It remains interesting that effects and also direction of effects varied among years (Table 2.1). In most years the effect of light level was not significant, but when it was, the direction of the relationship was variable, i.e. negative in some years and positive in another. Such variation in the correlation between light value and lay date is difficult to explain and the limited number of years do not allow for formal testing of the effect of other environmental variables on this relationship. Only for the stock dove, the negative relation between average first egg laying date and light emission as well as sky brightness seems to be apparent in all six years, but our sample size for this species was lowest of all, and thus this result needs to be treated with caution.

The estimated differences in lay date within the range of the light levels of our areas seem not very large in absolute sense, for example for pied flycatchers from ten days in 2014 to 15 days in 2010, but these differences in seasonal timing are biologically highly relevant. In comparison, pied flycatchers advanced their mean laying date following the advancement of the peak of abundance of nestling food due to increased spring temperatures over a period of 24 years by only 10 days (Both and Visser 2000).

With this study we aimed to get insight in how timing of avian breeding is influenced by the level of urbanization of an area. We used nocturnal light levels as a measure of urbanization and our study is correlative; we cannot be sure of any effects caused by light alone. Apart from light at night presumably affecting egg laying dates, temperature and food availability may be important factors too, and these are all related and increased in urban, compared to rural or natural environments (Partecke et al. 2005).

Here, we looked at timing of breeding, but not fledging success as this variable was measured less consistently by the volunteers collecting these data. To be able to draw conclusions on the implications of urbanization for wildlife, we need to include a measure of how well the birds are doing, for example by assessing reproduction and survival. It could be that increased (human-provided) food availability in urban areas forms an ecological trap (Schlaepfer et al. 2002) by

advancing lay date, but decreasing nest success because the available food may be less suitable for chicks (Chamberlain et al. 2009), whereas earlier laying dates in natural areas usually do lead to higher fitness (Visser et al. 2006).
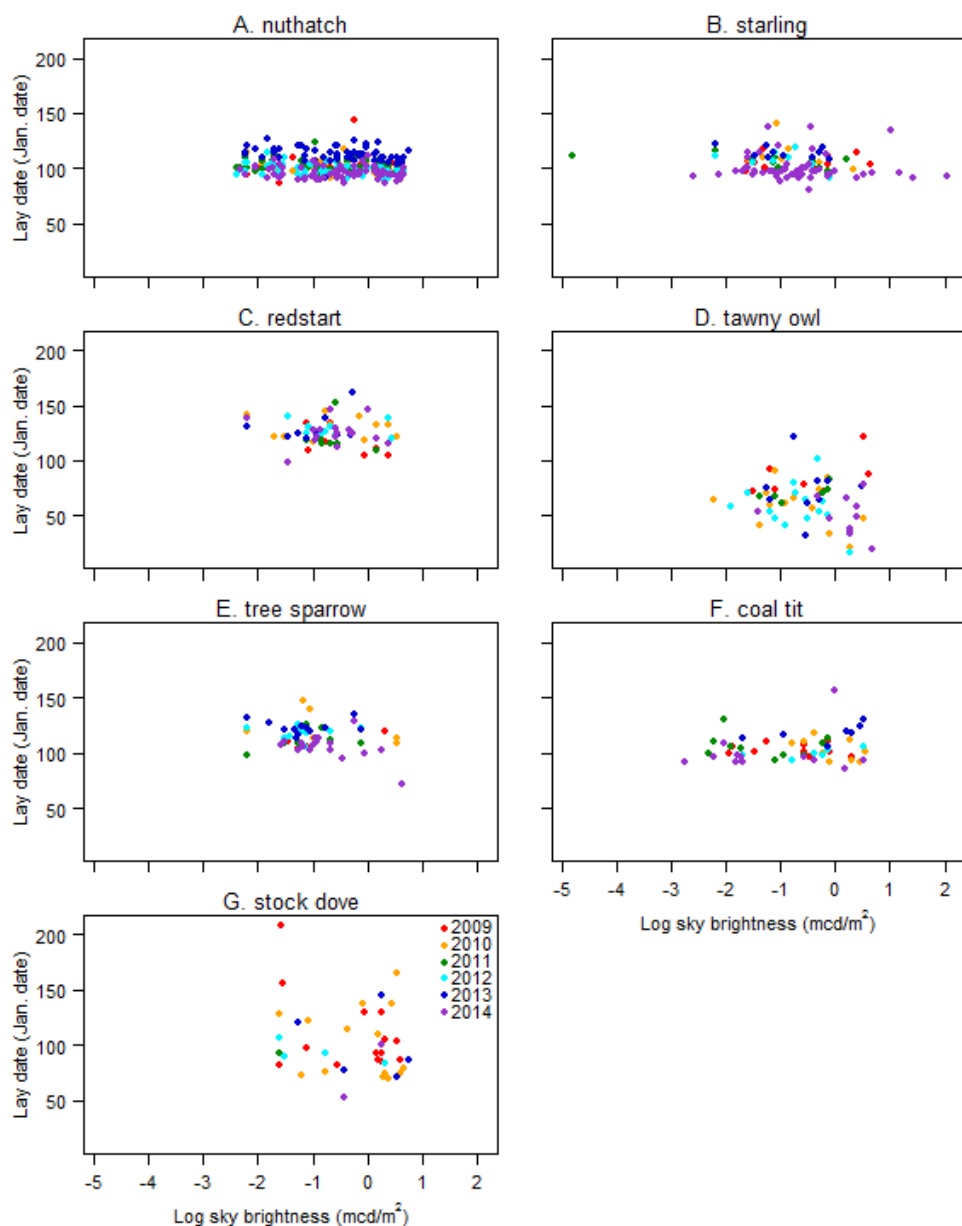
The 259 study areas from which we used the average first egg laying dates are distributed across all of the Netherlands (Figure 2.1), although it is clearly visible that we have much more data available for the least urbanized areas. Especially, we have few data from the Western part of the country which has a dense human population and high levels of light pollution. In general, we have very few data points in the larger cities and the heavily urbanized areas. This might explain the absence of a clear pattern in our dataset. Potentially, a much stronger correlation with urbanization is present, but will only emerge when the full range of light values is included and light values are better balanced in the dataset.

Therefore, we recommend expanding data collection on timing of avian breeding and avian fitness into more urban areas, and we aim to include more study areas in highly lighted areas in the NESTKAST project, in order to quantify impact of urbanization on wild bird species. In the Netherlands, one of the most urbanized countries with more than 90% of the population living in urban areas (United Nations 2014), city wildlife and urban ecology form an increasingly important part of nature conservation and thus it is important to measure consequences of urbanization for biodiversity.

Acknowledgements

Appendix

Figure 2.A1 Lay date versus log of zenithal sky brightness for species for which the interaction between light and year is not significant. Each dot indicates an average first egg laying date for a specific area in a specific year.

Chapter 3

Effects of nocturnal illumination on life-history decisions and fitness in two wild songbird species

Maaike de Jong
Jenny Q. Ouyang
Arnaud Da Silva
Roy H.A. van Grunsven
Bart Kempenaers
Marcel E. Visser
Kamiel Spoelstra

Abstract

The effects of artificial night lighting on animal behaviour and fitness are largely unknown. Most studies report short-term consequences in locations that are also exposed to other anthropogenic disturbance. We know little about how the effects of nocturnal illumination vary with different light colour compositions. This is increasingly relevant as the use of LED lights becomes more common, and LED light colour composition can be easily adjusted. We experimentally illuminated previously dark natural habitat with white, green and red light, and measured the effects on life-history decisions and fitness in two free-living songbird species, the great tit (*Parus major*) and pied flycatcher (*Ficedula hypoleuca*) in two consecutive years. In 2013, but not in 2014, we found an effect of light treatment on lay date, and of the interaction of treatment and distance to the nearest lamp post on chick mass in great tits but not in pied flycatchers. We did not find an effect in either species of light treatment on breeding densities, clutch size, probability of brood failure, number of fledglings and adult survival. The finding that light colour may have differential effects opens up the possibility to mitigate negative ecological effects of nocturnal illumination by using different light spectra.

3.1 Introduction

Light pollution has shown a worldwide increase in the last century, especially in the last six decades (Hölker et al. 2010), and artificial lighting of urbanised and rural areas continues to increase. 19% of the Earth's surface experiences nocturnal illumination from artificial sources and one-fifth of the world's population lives in areas where the Milky Way cannot be seen with the naked eye (Cinzano et al. 2001). Light pollution is considered a problem for many organisms, including humans; evidence for short-term negative effects of artificial light on several species is accumulating (Rich and Longcore 2006). Modern LED outdoor lighting allows for custom-built spectra, and adaptation of the light spectrum could be one of the options to reduce the effects of night time light pollution on ecosystems (Gaston et al. 2012).

One reason why light pollution has such a profound effect on organismal function may be that organisms have evolved under a natural light-dark cycle with high levels of light in daytime and very low levels of light at night. In birds, photoperiod is one of the most important factors determining daily activity patterns as well as seasonal timing. Their internal circadian and circannual clocks are entrained by light stimulation of photoreceptors to time physiological activities to the appropriate time of the day and year (Dawson et al. 2001). Artificial night lighting is hypothesized to affect the perceived photoperiod and thereby change the natural and temporal behaviour of birds, which in turn might affect their fitness (Farner 1964).

Research has only recently focused on changing light conditions at night and the understanding of the ecological consequences of light pollution is still limited. A well-known response of birds is attraction to artificial light, which causes high mortality of seabird fledglings due to fatal collisions and higher predation (Rodríguez et al. 2014), and of songbird nocturnal migrants due to exhaustion at light sources (Jones and Francis 2003). Other studies on bird populations in the wild have shown that the presence of street lights may cause several species to sing earlier at dawn (Nordt and Klenke 2013; Da Silva et al. 2014) and in the year (Da Silva et al. 2015), female blue tits (*Cyanistes caeruleus*) to advance egg laying (Kempenaers et al. 2010) and female great tits (*Parus major*) to increase chick feeding rates (Titulaer et al. 2012). In an experimental study in a controlled environment, nocturnal illumination advanced the reproductive physiology of blackbirds (*Turdus merula*) on a short term basis (Dominoni et al. 2013a), but suppressed reproductive activity in the long run (Dominoni et al. 2013d). An experimental study in a wild godwit (*Limosa limosa*) population revealed that early arriving godwits chose nest sites at greater distance from road lighting than late

arriving birds (de Molenaar et al. 2006). This relatively small set of studies all demonstrate rather short term effects of light pollution on the behaviour of birds. Experimental studies on the effect of light on life-history traits and fitness components in a field situation with no other anthropogenic disturbance are lacking (Spoelstra and Visser 2014).

The role of spectral composition in the impact of nocturnal illumination on avian behaviour is poorly studied (Musters et al. 2009), although the omission of specific colours could mitigate possible negative effects (Spoelstra and Visser 2014). Gonadal growth, a measure of reproductive readiness, is dependent on the wavelength of the light to which birds are exposed; longer wavelengths (red light) advance growth (Kumar et al. 2000b). Nocturnally migrating birds are disoriented by illuminated spots, especially with overcast skies, and removing red light from the spectrum makes this effect less pronounced (Wiltschko et al. 1993; Poot et al. 2008). A possible mechanism is that cryptochrome receptor molecules are dependent on short-wavelength light, which aligns with the wavelength dependency of magnetoreception observed in behavioural tests (the avian radical pair mechanism hypothesis; Liedvogel et al. 2007; Rodgers and Hore 2009).

In order to gain more knowledge on the effects of artificial night lighting on life-history decisions and fitness components of wild individuals of passerine birds, we studied their response to light at night during the breeding season. We make use of a unique, large scale, experimental set-up in the Netherlands, where we assess the effects of three different colours of street lighting on several species groups (Spoelstra et al. 2015). At our eight study sites, previously dark, natural habitat is experimentally illuminated with white, green or red light, in addition to a dark control. As a result of the altered perception of photoperiod due to the light at night, we expect birds that are breeding in illuminated territories to start laying eggs earlier compared to those in the dark. For light colour, we expect the strongest effect for white light, then red light (which is known to affect the reproductive system (which is known to affect the reproductive system; Kumar et al. 2000b), followed by green light. Although light at night may increase male fitness (Kempenaers et al. 2010), we have no clear expectations for effects towards different colours of nocturnal illumination on fitness components. If light attracts insects at night, resulting in higher insect density in illuminated areas in daytime, the fitness of insectivorous bird species may increase. However, light at night may adversely impact daily rhythms and reproductive physiology, and thereby decrease fitness. The experimental nature of our set-up gives the possibility to test the effects of nocturnal illumination independent of other anthropogenic disturbances normally associated with light at night.

3.2 Methods

3.2.1 Experimental set-up

At eight sites in the Netherlands (Spoelstra et al. 2015), we illuminate previously dark natural areas with street lamps (intensity 8.2 ± 0.3 lux, measured directly under the lamp at ground level), from sunset until sunrise. Each site has four transects with five lamp posts with LED lights, each transect with one light colour treatment; Fortimo white, ClearSky green and ClearField red light (Philips, Amsterdam, The Netherlands), and a dark control (poles without lamps). Within each site, each transect was randomly assigned a light treatment. All three lamp types emit full spectrum light, however green lamps have an increased blue and reduced red and red lamps have an increased red and reduced blue emission (for details on the spectral power of the light see Spoelstra et al. 2015). The intensity of the light at ground level at all transects is standardized for human vision (in lux), such that the light of the three different colours is perceived by humans as equally intense. The sensitivity spectrum of birds differs from that of humans, most birds perceive colours through four single cone types. The intensity of the treatments is therefore different for birds. The ability of birds to see UV light (Bennett and Cuthill 1994; Osorio and Vorobyev 2008) does not contribute to this difference as the UV emission of our lights is negligible. We chose to standardize the intensity at all transects in lux, because the street lamps we have placed at our study sites are eventually intended for road lighting for human purposes.

Sites consist of coniferous, deciduous or mixed forest edge habitat where four transects, each consisting of five lamp posts, were placed perpendicular to the forest edge in 2012, and at one of the sites in 2013 (Figure 3.1 and Spoelstra et al. 2015). A large variety of species groups is yearly monitored at these sites, (see also Spoelstra et al. 2015). In order to study the breeding ecology of cavity-breeding passerines, at each site, 36 bird nest boxes (diameter entrance hole 32 mm) were placed in the forest, in the year the lamp posts were set up (288 in total). Our sites have few natural cavities. The placement of nest boxes follows a standardized pattern, in order to test the effects of light on individuals nesting at different distances from the lamp posts (see Figure 3.1).

All data were collected during the springs of 2013 and 2014. The nest boxes were occupied by breeding pairs of four species; great tit, 97 broods in 2013 and 138 broods in 2014, pied flycatcher (*Ficedula hypoleuca*), 49 and 67 broods respectively, blue tit, 15 and 22 broods, and coal tit (*Periparus ater*), two broods in 2013 and one in 2014. Here, we report on the life-history traits and fitness components for the great tit, a small, 18 g resident songbird, and the pied

flycatcher, a 12 g trans-Sahara migrant songbird (sample sizes for blue tits and coal tits were too small to conduct meaningful statistical analysis).
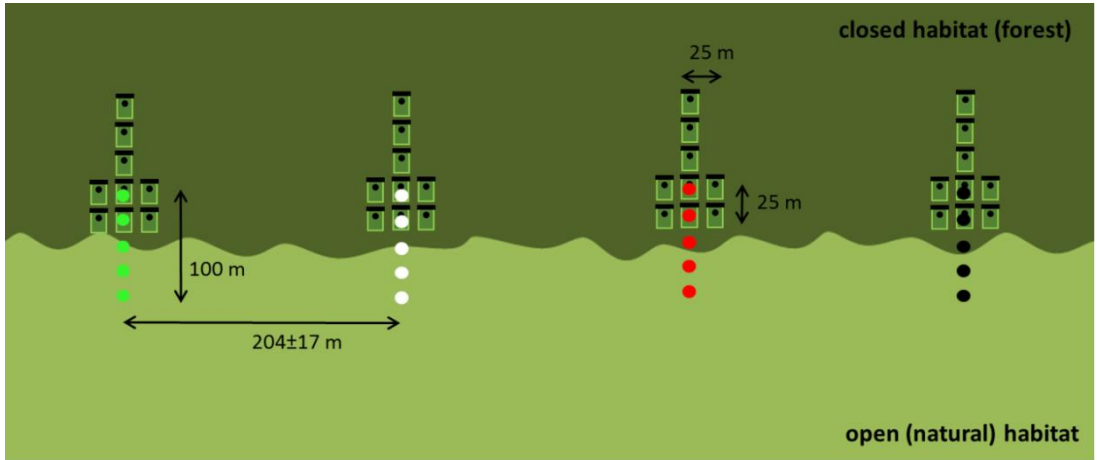
Figure 3.1 Schematic overview of the set-up of one study site, which is replicated eight times. Five lamp posts are placed in transects perpendicular to the forest edge. Within a site, orientation of transects was constant. Distance between transects is variable and depends on the local situation. Each transect was randomly assigned to one of the four light treatments, here green, white, red and dark respectively. In each transect nine nest boxes were attached to trees at 1.6 m height and at approximately 25 m distance from each other (dependent on the nearest tree). Orientation of the nest box opening was always towards the forest edge.

3.2.2 Field methods

Nest boxes were checked twice weekly from the end of March until the end of the breeding season (end of June / early July) in 2013 and 2014. We recorded nest stage, number of eggs and species. In this study, we only used data from first broods for both species; both replacement broods and second broods were excluded. All clutches that started more than 30 days for great tits, or 22 days for pied flycatchers, after the first clutch in that site and year were considered to be replacement clutches. First egg laying dates were calculated on the assumption that one egg is laid per day. The number of eggs after clutch completion (clutch size) and exact egg hatching dates were recorded. During the nestling stage, chicks were ringed with a numbered aluminium ring (eight days after hatching in great tits, six days after hatching in pied flycatchers). The mass of the chicks, a measure of fledgling quality (Verboven and Visser 1998), was recorded using a digital scale (nearest 0.1 g, 15 and 13 days after hatching in great tits and pied flycatchers, respectively). Adults were caught in the nest box using a spring trap

and ringed with a numbered aluminium ring (great tits: 8-9 days (2013) and 10-12 days (2014) after hatching; pied flycatchers: 6-7 days (2013) and 9-11 days (2014) after chick hatching). Nests were checked after the chicks fledged, and the number of fledglings is the number of chicks that left the nest.

3.2.3 Statistical methods

In all models, we fitted the interaction between light treatment (a factor with four levels; dark, green, red and white) with the distance of the nest box to the nearest lamp post, because we expected the effect of light to decrease with light intensity (see Appendix Figure 3.A1 for the relation between light intensity at nest box level and distance to the nearest lamp post). We also included site (a factor with seven levels in 2013 and eight in 2014) as a random effect to account for between-site differences. Additionally, in the models for fledgling mass we added brood size (the number of chicks that hatched) as an explanatory variable and nest box as a second random effect, to account for common environment effects of chicks raised in the same brood. Sex was used as explanatory variable in the models for adult survival. We analysed the data for both species and both years separately.

Data on settlement of the breeding pairs was analysed using a generalized linear mixed-effects model (GLMM) with binomial error structure and occupancy of the nest box (0 = not occupied; 1 = occupied) as response variable. Egg laying dates (first egg; in April date, May 1 = April 31) and clutch sizes (number of eggs) were analysed using linear mixed-effects models (LMMs). Fledging success was computed in two steps, as the distribution of the number of fledged chicks for the great tits was strongly zero-inflated. First, we analysed the probability of brood failure (0 = at least one chick fledged; 1 = no chicks fledged) in a GLMM with binomial errors. Second, we analysed the number of chicks fledged excluding brood failures in a LMM, following (Reed et al. 2013). Pied flycatchers had very few nests that failed (10 out of 108), therefore we only analysed the number of chicks that fledged excluding brood failures (LMM). Fledgling mass was analysed using an LMM and adult survival using a GLMM with binomial errors (0 = found breeding in 2013 but not in 2014; 1 = found breeding in 2013 and 2014). All statistical analyses were done using R version 3.1.1 (R Development Core Team 2014) with a significance level of $\alpha = 0.05$.

3.3 Results

Our light treatment had no effect on the probability of nest box occupancy by great tits or pied flycatchers. In great tits, nest boxes closer to the lamp posts were occupied less often in both 2013 and 2014; this effect was the same in the dark control transects (Table 3.1).

In 2013, there was a significant effect of light treatment on laying date of great tits, birds in green and white illuminated transects laid their eggs on average earlier than those in the dark control (Figure 3.2A & Table 3.1). In 2014, however, there was no effect of light treatment on laying date. In pied flycatchers, we found no effect of light treatment on lay date in either years (Figure 3.2B & Table 3.1). Clutch size in both great tits and pied flycatchers was not affected by light treatment, but in 2013 great tits laid larger clutches further away from the poles, independent of treatment (Table 3.1).

Light treatment did not affect the probability of brood failure (no chicks fledged) or the number of chicks fledged (if at least one chick fledged) in great tits in either year (Figure 3.3A & Table 3.1). In 2014, great tits breeding further away from the poles fledged less offspring, again independent of treatment. In the pied flycatcher, the number of chicks fledged was also not affected by light treatment (Figure 3.3B & Table 3.1).

In 2013, but not in 2014, fledgling mass in great tit broods was explained by the interaction between treatment and distance to the nearest lamp post, in combination with brood size (see for estimates Table 3.1). For pied flycatchers there was no treatment effect on fledgling mass in either year (Table 3.1).

The probability of survival from breeding season 2013 to breeding season 2014 did not differ between light treatments in both great tits and pied flycatchers (Table 3.1). Some of the surviving females and males moved from one light treatment to another between years, but without any clear pattern (out of 18 surviving female great tits eight moved; out of 12 surviving great tit males one moved; out of six surviving female pied flycatchers one moved; out of 12 surviving male pied flycatchers eight moved).

3.4 Discussion

We assessed the effects of light at night with different spectral composition on the breeding biology and fitness components of two wild songbird species. The effect of light treatment on timing of egg laying, one of the life-history traits, was not consistent across species and years. Fledgling production, an important component of fitness, was not affected by light at night in both species, fledgling mass was, but only for one species in one year. Thus, we did not show clear, unidirectional effects of experimental nocturnal illumination on fitness.

Settlement of our birds at the study sites was not affected by light treatment, but occupancy rates for great tits were higher further away from the lamp posts, also in the control treatment. Due to the spatial pattern of our nest boxes, the density of nest boxes decreases with increasing distance to the lamps. Great tits usually defend territories larger than 25 m radius (the distance between our nest boxes) during the breeding season (Both and Visser 2000), and thus each territory will contain more than one nest box, leading to the observed pattern of increased occupancy rates further away from the lamp posts at all four treatment groups. In contrast, pied flycatchers defend just the area directly around their nest box (Alatalo and Lundberg 1984), which may explain the absence of an effect of distance on occupancy rate observed in this species. We found no effect of artificial light at night on clutch size in either species.

Table 3.1 (*On the next page.*) Results of the generalized linear mixed-effects models (GLMM) and linear mixed-effects models (LMM) on seven response variables, for great tits and pied flycatchers, in 2013 and 2014. For each term the numerator and denominator degrees of freedom (df), the F test statistic (F) and the significance level (P) are given.

[1] P values are in bold when considered significant (<0.05). For the significant terms the estimate is given behind the P value, between brackets.
[2] For comparisons of LMM an F test was calculated according to the approach of Kenward and Roger, GLMM were compared using parametric bootstrap methods where a number of simulations of the Likelihood Ratio Test statistic are generated (Halekoh and Højsgaard 2014). Therefore, no degrees of freedom or F test statistic are given for the GLMM.
[3] Estimates for lay date for each treatment: dark 33.3, green 29.0, red 33.2 and white 29.5.
[4] Pied flycatchers had very few nests that failed, therefore we only analysed the number of chicks that fledged excluding brood failures.
[5] Because we found a significant interaction effect of treatment by distance to the nearest lamp post on mass of great tit chicks in 2013, we did not calculate P values for the individual fixed effects.
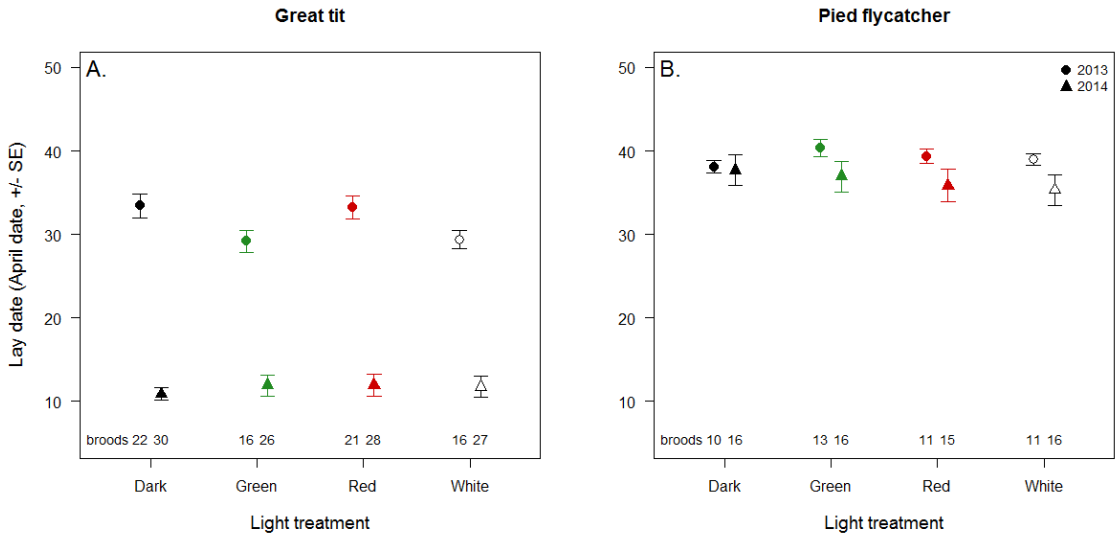[6] Estimates for chick mass for each treatment: in dark chick mass = 21.0 – 0.022 * distance, in green 18.6 – 0.004 * distance, in red 18.1 + 0.024 and in white 16.6 + 0.06 * distance.
[7] Adult survival to the next breeding season could only be calculated for birds breeding in 2013.

Table 3.1 (*Continued.*)

		2013			2014	
				Great tit		
	df	F	P[1]	df	F	P[1]
Occupancy						
GLMM (random: Site)		(n=252)			(n=288)	
Treatment	[2]		0.61	[2]		0.98
Distance to lamppost			**0.02 (0.02)**			**0.02 (0.03)**
Treatment : Distance to lamppost			0.17			0.32
Lay date						
LMM (random: Site)		(n=75)			(n=111)	
Treatment	3, 68.04	2.84	**0.04** [3]	3, 103.95	0.17	0.92
Distance to lamppost	1, 69.81	0.34	0.56	1, 105.47	1.56	0.22
Treatment : Distance to lamppost	3, 63.81	0.46	0.71	3, 100.42	0.40	0.75
Clutch size						
LMM (random: Site)		(n=66)			(n=104)	
Treatment	3, 59.39	0.37	0.77	3, 96.13	0.44	0.72
Distance to lamppost	1, 63.44	4.32	**0.04 (0.02)**	1, 97.32	0.06	0.81
Treatment : Distance to lamppost	3, 54.86	1.31	0.28	3, 91.45	0.30	0.83
Probability of brood failure						
GLMM (random: Site)		(n=75)			(n=111)	
Treatment	[2]		0.71	[2]		0.71
Distance to lamppost			0.53			0.45
Treatment : Distance to lamppost			0.07			0.42
Number of fledglings (if ≥ 1)						
LMM (random: Site)		(n=41)			(n=84)	
Treatment	3, 35.59	0.97	0.42	3, 73.60	0.71	0.55
Distance to lamppost	1, 33.70	0.02	0.89	1, 79.13	4.16	**0.04 (-0.01)**
Treatment : Distance to lamppost	3, 32.03	0.45	0.72	3, 70.05	1.11	0.35
Chick mass						
LMM (random: Site & Nest box)		(n=214)			(n=535)	
Treatment	[5]			3, 72.30	0.09	0.97
Distance to lamppost				1, 79.35	0.01	0.93
Brood size	1, 32.64	21.57	**<0.001 (-0.55)**	1, 86.46	0.76	0.38
Treatment : Distance to lamppost	3, 29.79	3.28	**0.03** [6]	3, 69.96	0.43	0.73
Adult survival						
GLMM (random: Site)		(n=99)				
Treatment	[2]		0.27	[7]		
Distance to lamppost			0.67			
Sex			0.28			
Treatment : Distance to lamppost			0.27			

Table 3.1 (*Continued.*)

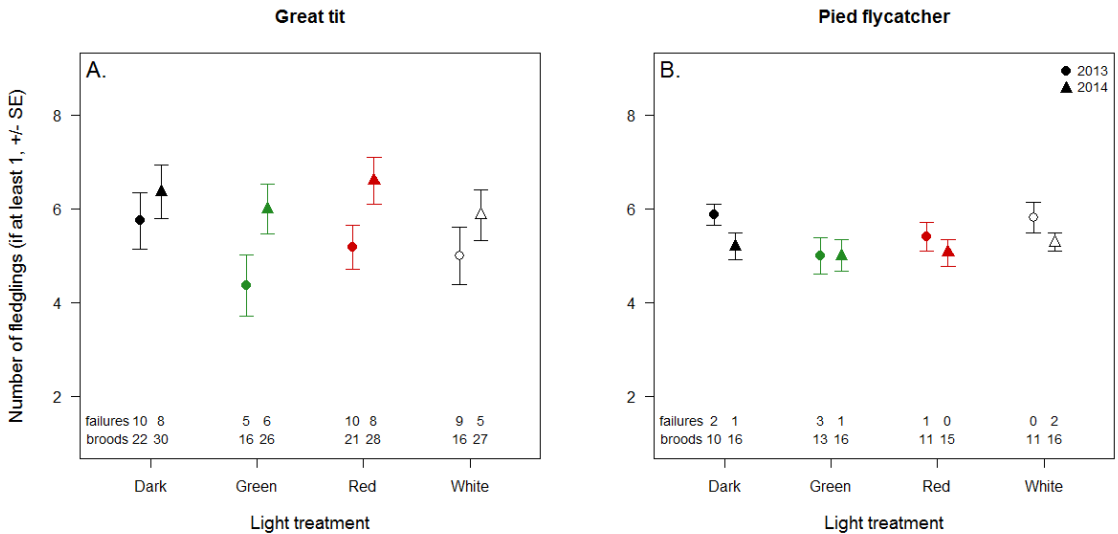|	Pied flycatcher					
	2013			2014		
	df	F	P[1]	df	F	P[1]
Occupancy						
GLMM (random: Site)		(n=252)			(n=288)	
Treatment	[2]		0.90	[2]		1.00
Distance to lamppost			0.94			0.47
Treatment : Distance to lamppost			0.91			0.80
Lay date						
LMM (random: Site)		(n=45)			(n=63)	
Treatment	3, 37.52	1.10	0.36	3, 55.14	0.36	0.79
Distance to lamppost	1, 39.92	0.01	0.94	1, 59.43	0.31	0.58
Treatment : Distance to lamppost	3, 35.36	0.73	0.54	3, 53.87	0.08	0.97
Clutch size						
LMM (random: Site)		(n=44)			(n=63)	
Treatment	1, 41.90	0.14	0.71	3, 56.01	1.20	0.32
Distance to lamppost	1, 38.94	0.05	0.82	1, 56.64	0.03	0.86
Treatment : Distance to lamppost	3, 34.25	0.43	0.74	3, 53.87	0.81	0.50
Probability of brood failure						
GLMM (random: Site)						
Treatment	[4]			[4]		
Distance to lamppost						
Treatment : Distance to lamppost						
Number of fledglings (if ≥ 1)						
LMM (random: Site)		(n=39)			(n=59)	
Treatment	3, 30.92	2.03	0.13	3, 52.55	0.20	0.89
Distance to lamppost	1, 31.16	0.07	0.79	1, 53.88	0.00	0.96
Treatment : Distance to lamppost	3, 27.74	0.72	0.55	3, 49.67	2.10	0.11
Chick mass						
LMM (random: Site & Nest box)		(n=199)			(n=281)	
Treatment	3, 29.97	1.33	0.28	3, 46.98	0.65	0.59
Distance to lamppost	1, 28.75	0.27	0.61	1, 49.26	0.08	0.78
Brood size	1, 31.64	4.11	0.05	1, 53.07	0.36	0.55
Treatment : Distance to lamppost	3, 24.40	0.29	0.83	3, 44.13	1.00	0.40
Adult survival						
GLMM (random: Site)		(n=80)				
Treatment	[2]		0.90	[7]		
Distance to lamppost			0.38			
Sex			0.18			
Treatment : Distance to lamppost			0.36			

Figure 3.2 Average first egg laying dates (April date) for each light treatment, for great tits (A) and pied flycatchers (B; see also Table 3.1). Circles are 2013, triangles are 2014 data and error bars show +/- 1 standard error. Sample sizes (number of broods) are indicated above the x axis for each treatment in each year. Average first egg laying date in 2013 was 31.9 for great tits and 39.2 for pied flycatchers, and in 2014 11.2 and 35.5 respectively.

Our findings on seasonal timing of great tits in 2013 are in line with the advancement in lay date of blue tits in illuminated territories reported by Kempenaers et al. (2010). However, the effect of artificial light on lay date was not consistent in our study. One key difference between the study by Kempenaers et al. and ours is that our study is experimental and thus treatments only differ in the level of light at night, whereas in Kempenaers et al. differences in light levels may be correlated with other anthropogenic factors (e.g. lighted territories were also closer to human habitation).

Day length is a strong cue in timing of the start of egg laying (Lambrechts et al. 1997) and light at night could lead to birds perceiving a longer photoperiod. In 2013, light treatment had a significant effect on the start of breeding of great tits. In 2014, when spring was warmer and birds laid much earlier, there was no effect of light at night. An explanation for this difference could be that in cold years with a late season, such as 2013, photoperiod may play a more pronounced role in the onset of egg laying than in warm years with an early season (Gienapp et al. 2005), such that artificial night lighting would only affect laying date in the former. Obviously, 2013 and 2014 differed in more than just their mean spring temperature, but it is well known that temperature and photoperiod are the most

important environmental variables affecting lay date. We could not identify clear differences between individual light colours, but the effect of light treatment may be due to the advancement of lay date by exposure to white and green light. If this is indeed the case, this effect is contradictory to our expectation that red but not green light advances breeding. However, the effects of red light (Kumar et al. 2000b) were reported for gonadal growth, whereas the timing of actual egg laying may be affected in a different way. Clearly, data from more years are needed to reveal an interactive effect of light at night and spring temperatures. Laying date of pied flycatchers was not affected by nocturnal illumination, which may be related to their timing of migration; they arrive at their breeding grounds few days before the first eggs are laid and so the exposure to the light at night might not be long enough to affect timing of egg laying. Also, different spectra may have differential effects on different species, because of species specific spectral sensitivity (Vorobyev et al. 1998).

Figure 3.3 Average number of fledglings of broods that fledged at least one chick, for each light treatment, for great tits (A) and pied flycatchers (B; see also Table 3.1). Circles are 2013, triangles are 2014 data and error bars show +/- 1 standard error. Number of failed broods (zero fledglings, failures) and sample sizes (number of broods) are indicated above the x-axis for each treatment in each year.

Artificial night lighting did not significantly affect reproductive success in either species. In pied flycatchers, fledgling mass was not affected by artificial light at night, however in great tits chick mass depended on treatment in relation to distance to lamp posts in 2013, but not in 2014. There are thus no strong indications that fledgling production or fledgling quality are affected by artificial

night lighting. Nocturnal illumination did not influence the survival rates from breeding season 2013 to 2014 in either species, but the amount of data on adult survival is limited.

Fitness effects of nocturnal illumination in birds have, as far as we know, never been studied experimentally in the field. We present the first results on this here, which suggest that the effects of artificial night lighting on breeding success are absent or small. This study is one of the first to document no, or very little, effect of artificial light at night on individual organisms (see Gaston et al. 2015). Although we have data from 288 nest boxes over two years, the dataset we present is still relatively small so that only relatively strong effects would have been detected. Clearly, more data is needed to draw conclusions on fitness effects and ultimately contribute to evidence-based advice on nature friendly outdoor lighting.

Our study is experimental in the sense that we started illuminating a formerly dark forest and kept part of it dark. We placed the same number of nest boxes in all transects using the same pattern. However, it was not possible to control for settlement differences, since individual birds were free to choose whether or not to start breeding near the lamp posts. This choice opens the possibility that a non-random selection of the population breeds in nest boxes under light at night. However, we did show that the breeding density of birds did not differ between light treatments, and birds that survived from 2013 to 2014 did not move to a particular light colour or away from the illuminated area to the dark control.

Because the light intensity quickly decreases with increasing distance from the lamp posts, there are ample dark places relatively close to our nest boxes. The nest boxes furthest away from the lamps are not different from those in the dark transects in terms of light intensity. Birds breeding in the illuminated nest boxes thus have the opportunity to escape the direct effect of light, by moving away from it or by being inside the nest box. This behavioural modulation could also explain the absence of strong effects on breeding success. We are currently doing measurements to determine how much light adult birds actually perceive at our experimental field sites. Chicks in nest boxes receive very low light levels (typically below 0.05 lux), even if these boxes are directly under the lamps. We want to stress however that the light levels used in our set-up are representative for outdoor lighting of, for example, roads.

Apart from direct effects of nocturnal illumination, for instance changing the perception of day length which relates to seasonal timing, there can also be indirect effects. Nocturnal illumination can for example affect insect abundance

(Eisenbeis and Hassel 2000), which is the major food source for our birds during the breeding season. In our experimental set-up, it is not possible to separate these direct from indirect effects, and additional experiments in a controlled environment are necessary to identify causal relationships.

In the current study, we show that experimental nocturnal artificial light in the field can affect timing of egg laying and fledgling mass, a predictor of recruitment, but only in one species and in one year. For most life-history variables and fitness components we found no effects. Given the widespread use of artificial light at night, many breeding birds are exposed to light levels similar to those in our study. The non-consistent effects that we found indicate the need for long term studies. Furthermore, if the magnitude and direction of possible effects depend on the spectral composition of the light, that could open up the possibility to mitigate specific ecological consequences with the use of coloured nocturnal illumination.

Light pollution is considered a global biodiversity threat (e.g. Hölker et al. 2010). Evidence of a wide variety of effects on behaviour of birds is accumulating, but many important questions remain to be answered; does light at night matter on a larger scale, are terrestrial breeding bird populations doing poorly in areas with more night-time illumination? The experimental design described here creates the opportunity to answer these questions and to do so we will continue to record data on nest box breeding birds as well as all other birds present at our sites (as described in Spoelstra et al. 2015), during the coming years.

Ethical statement

This study was carried out with the approval of the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences.

Authors' contributions

The set-up and design of the study origins from elaborate discussion between MEV, KS and RHAvG. KS and RHAvG established the field sites. Data were collected by MdJ, JQO and ADS and analysed by MdJ, MEV and KS with input from RHAvG, ADS and BK. The paper was drafted by MdJ, MEV and KS and JQO, ADS, RHAvG and BK have contributed to the writing.

Funding

Competing interests

We declare no competing interests.

Acknowledgements

Appendix

Light level at nest-boxes
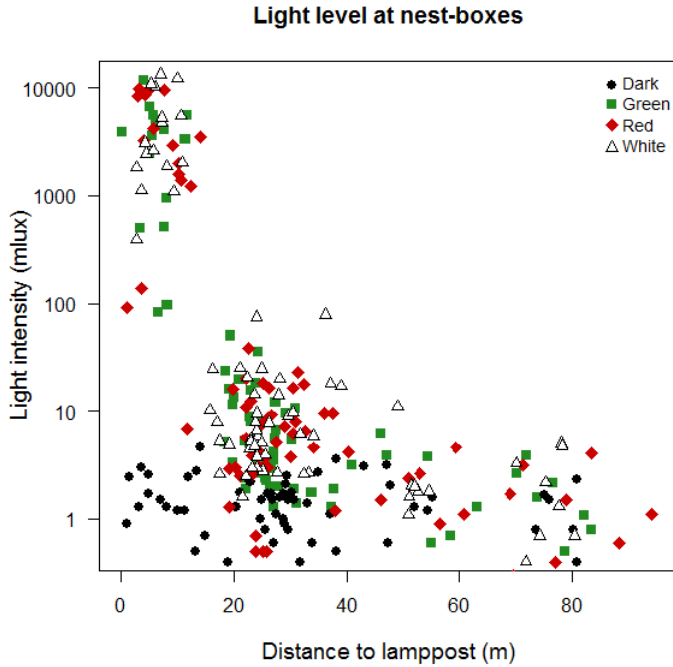
Figure 3.A1 Light intensity (mlux) at nest-box entrance level in relation to distance to the nearest lamp post (m) for all four light treatments (filled black circles are nest-boxes in the dark treatment, filled green squares are nest-boxes in the green treatment, filled red diamonds are nest-boxes in the red and open triangles are nest-boxes in the white light treatment). We present the average light intensity value of measurements in four directions at each nest-box entrance; upward, forward, to the left and to the right. Measurements have been done with a calibrated illuminance meter, LMT B 360 (LMT Lichtmesstechnik GmbH, Berlin, Germany).

Chapter 4

Cheaters like it dark: female great tits breeding in illuminated areas are more faithful

Maaike de Jong
Koosje P. Lamers
Mark Eugster
Jenny Q. Ouyang
Arnaud Da Silva
A. Christa Mateman
Roy H.A. van Grunsven
Marcel E. Visser
Kamiel Spoelstra

Abstract

Light pollution is increasing worldwide and significantly affects animal behaviour. In birds, these effects include advancement of morning activity and onset of dawn song, which may affect extra-pair paternity. Advanced dawn song of males may change the motivation of females to engage in extra-pair copulations, and the earlier activity onset may affect the males' mate guarding behaviour. Earlier work showed an effect of light at night on extra-pair behaviour, but this was in an area with other anthropogenic disturbances. Here, we present an experimental study on effects of light at night on extra-pair paternity of great tits (*Parus major*), in two years. Previously dark natural areas were illuminated with white, red and green LED lamps and compared to a dark control. In 2014, the proportion of extra-pair young in broods increased with distance to the red and white lamps, but decreased with distance in the dark control. In 2013, we found no effects on the proportion of extra-pair young. The total number of offspring sired by a male, was unaffected by artificial light at night, suggesting that the observed changes in female fidelity in pairs breeding close to white and red light do not translate into fitness benefits for the males of these pairs. We thus show that artificial light disrupts the natural patterns of extra-pair paternity, and possibly negates potential benefits of extra-pair copulations, as females breeding in the light have fewer extra-pair offspring. Our findings imply that artificial light at night disrupts sexual selection processes in wild birds.

4.1 Introduction

The loss of dark nights across the world forms a biodiversity threat (Hölker et al. 2010), and the amount of artificial night lighting is predicted to continue to rise in the future (Cinzano et al. 2001). We are increasingly uncovering ecological consequences of light pollution (Rich and Longcore 2006; Gaston et al. 2015). In animals this is partly because their behaviour has evolved to be synchronized with the natural light-dark cycle; photoperiod drives circannual and circadian rhythms (Dawson et al. 2001). Evidence of short-term effects of nocturnal illumination on animal behaviour and physiology is accumulating (Swaddle et al. 2015). In birds, light at night affects daily timing of behaviour. Onset of daily activity of blackbirds (*Turdus merula*) advances in urban compared to rural sites, and birds exposed to higher light intensities become active earlier in the morning (Dominoni et al. 2014). An experimental study in great tits (*Parus major*) showed that birds advance their daily rhythm when exposed to light at night in a dose-dependent manner (de Jong et al. 2016). Also, artificial light affects dawn song: blackbirds in urban areas sing earlier due to anthropogenic noise and light (Nordt and Klenke 2013), and several songbird species sing earlier at dawn and in some cases, later at dusk (Miller 2006; Da Silva et al. 2014).

The onset of the dawn chorus may be a cue for male quality in some songbird species. Earlier singing blue tits (*Cyanistes caeruleus*) have more mating partners and are more likely to gain extra-pair offspring (Poesel et al. 2006). Male Eastern kingbirds (*Tyrannus tyrannus*) singing earlier sire more extra-pair young as well (Dolan et al. 2007). The onset of a male's dawn song thus may correlate with the choice of a female for extra-pair mating partners; earlier singing males may be more attractive and hence sire more extra-pair chicks. Additionally, an early singing male's social partner may be less eager to copulate with extra-pair males. The presence of artificial light at night may disrupt this natural cue of attractiveness by prompting birds living in illuminated territories to sing earlier (Kempenaers et al. 2010; Nordt and Klenke 2013; Da Silva et al. 2014), and thereby affecting female mate choice and extra-pair mating dynamics.

Another mechanism by which artificial light at night could affect extra-pair behaviour of a female is via her male's mate guarding. In great tits, the male sings near the nest box in the early morning and, once his partner emerges, subsequently copulates with her (Mace 1987). However, females mostly engage in extra-pair copulations at the peak of their fertility, and emerge earlier from their nest box at this time (Halfwerk et al. 2011; Schlicht et al. 2014). This suggests that great tit females actively seek extra-pair fertilizations by sneaking away before their social male becomes active. If the onset of activity of the social male is

advanced by light at night (Dominoni et al. 2014; de Jong et al. 2016), the male may be more successful at guarding his female partner and preventing her from engaging in extra-pair copulations.

The first evidence of an effect of artificial light on the extra-pair success of male songbirds was found by Kempenaers et al. (2010). Male blue tits that occupied illuminated forest-edge territories acquired more extra-pair mates, compared to males breeding in non-illuminated forest and forest-edge territories. This effect may be linked to the advancement of dawn song by light, which was found in the same area for the same species. The study by Kempenaers et al. shows the potential effect of light at night on extra-pair partner choice; however, these effects were observed around pre-existing light sources along a street in a suburban residential area. Therefore, the effects may be confounded with other anthropogenic disturbances associated with light.

In the current study, we investigate the effects of experimental light at night on extra-pair behaviour of great tits in the absence of other disturbances. Previously unlit areas were illuminated with white, red, and green LED lamps, and compared to a dark control (Spoelstra et al. 2015). In two consecutive years, we determined the parentage of great tit offspring and tested for an effect of light at night and distance to the light on the occurrence of extra-pair paternity (proportion of extra-pair young in a brood) and male reproductive success (total number of offspring sired). Following the two possible mechanisms described above, we expect females breeding in the light to have less extra-pair copulations (and thus fewer extra-pair offspring in their brood) and males from illuminated territories to sire more offspring (by siring more extra-pair offspring and losing less paternity in their own brood).

4.2 Methods

4.2.1 Experimental set-up
We illuminated previously dark natural areas with transects of street lamps (8.2 ± 0.3 lux at ground level) of three different colours (green, red and white LED light) in addition to a dark control (poles without lamps). Two areas contained two sites (eight transects) and four areas one site (four transects). Light treatment was randomly assigned to transects within sites. Areas are forest-edge habitat and lights were on from sunset to sunrise in five areas since 2012, and in one area since 2013. Nine bird nest boxes (diameter entrance hole 32 mm) were placed at each transect, following a standard pattern. For more details about the experimental set-up, the field sites and the spectral composition of the light, see

de Jong et al. (2015) and Spoelstra et al. (2015). All data were collected during the springs of 2013 and 2014.

4.2.2 Field methods

Nest boxes were checked twice weekly from the end of March until the end of the breeding season. We only used data from first broods of great tits (for details see de Jong et al. 2015). Samples for DNA-analysis were collected by taking blood samples from the heel vein of chicks two to four days after hatching, and by storing dead chicks and unhatched eggs. We sampled offspring and adults of 55 first broods in 2013, and of 94 first broods in 2014 (see Table 4.1); the number of broods in different treatments was similar (see Table 4.2). Adults were caught in the nest box using a spring trap (8–12 days after egg hatching), sexed, ringed with a numbered aluminium ring if unringed and a blood sample was taken from the brachial vein. Blood samples were stored in Cell Lysis buffer (Qiagen, Redwood City, USA). For the 2014 adult samples, plasma was separated first, and red blood cells were frozen and later transferred to Cell Lysis buffer.

4.2.3 Genetic analysis

96-well genomic DNA extraction of blood and tissue samples was performed with a Favorgen kit (Favorgen Biotech Corporation, Ping-Tung, Taiwan) as described in the manufacturer's user manual. PCR was performed as described by Saladin et al. (2003) using five microsatellite DNA loci; PmaTAGAn71, PmaGAn27, PmaTGAn33, PmaC25 and PmaD105. Separation of the PCR fragments took place using an ABI 3130 Genetic Analyzer (Thermo Fisher Scientific, Waltham, USA). The capillary electrophorese results of the ABI were analysed with the software GeneMapper 5.0 (Thermo Fisher Scientific, Waltham, USA) that determined the sizes of the amplification products.

4.2.4 Paternity analysis

Paternity analyses were performed with the likelihood-statistics program Cervus version 3.0.7 (Field Genetics Ltd, London, UK; Kalinowski et al. 2007). All analyses in Cervus were performed per area and year. Allele frequencies were calculated and none of the loci deviated from Hardy-Weinberg equilibrium (one of the assumptions of Cervus). The combined exclusion probability for the microsatellite markers was 0.98 (averaged over areas) in both 2013 and 2014. Individuals were categorized as within-pair (WP) or extra-pair (EP) offspring by comparing their genotype to that of the mother and social father. An individual was categorized as EP if one or more loci mismatched and Cervus-based analyses did not recognize the social father as the most likely father. For 3.4% of the offspring in 2013 and 2.2% in 2014 it was not possible to categorize the individual as WP or EP (see also Table 4.1). When offspring was EP, we compared its genotype to those of all

potential fathers sampled at the same area in both years. Critical values were calculated using the following parameters in Cervus: 10000 cycles, 98% of loci typed, error rate 0.01%. The 2013 offspring have been sexed using Griffiths' method (Griffiths et al. 1998) and fledged males were added to the 2014 analysis in order to increase the chances of identifying the genetic fathers for the 2014 offspring. Parentage was assigned to chicks with parent combination matches of 95% confidence. The methods used for paternity analysis are described in more detail in van Oers et al. (2008). We found two broods with only EP offspring, both at the same site (Voorstonden) where breeding density of great tits was very high and the proportion of EP young in nests was generally high (on average 29%).

4.2.5 Statistical analysis

All statistical analyses were performed using R v. 3.1.2 (R Development Core Team 2014) with a significance level of α=0.05. To investigate possible effects of artificial light at night on extra-pair behaviour of great tits at our experimental study areas, we analysed two response variables. First, we modelled the proportion of EP chicks in a brood (*cbind* number of EP over number of WP), using a generalized linear-mixed-effects model (GLMM) with binomial error distribution and logit link function. Then, we modelled the total number of offspring that was sired by a male (own WP offspring in social brood and EP offspring elsewhere), using a GLMM with Poisson error distribution and log link function. In both models, we fitted the interaction between light treatment (a factor with four levels: dark, green, red and white), the distance of the nest box to the nearest lamp post, because we expected the effect of light to decrease with light intensity, see de Jong et al. (2015), and year (a factor with two levels: 2013 and 2014). We also included area (a factor with five levels in 2013 and six in 2014) as a random effect to account for between-area differences, and male identity (social father of a brood), to account for double measurements of the same males in both years. We found 19 males breeding in 2013 as well as in 2014, of which only three occupied the same nest box. Because the light with distance with year interaction was significant, we analysed both years separately. Backward selection was used in both analyses, until only significant terms were left; the term with the highest *p*-value was taken out of the models first.

Ethical statement

This study was carried out under license NIOO 10.07 of the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences.

Table 4.1 Number of broods, adults and offspring, for both years and in total. All caught adults were genotyped. The number of not sampled offspring was negligibly small. Number of genetic fathers identified is for the total number of extra-pair offspring.

	Broods	Sampled females	Sampled social males	Sampled offspring	Genotyped offspring	Within-pair offspring	Extra-pair offspring	Genetic (extra-pair) father identified
2013	55	50	47	403	380	326	41	9
2014	94	88	85	802	775	640	118	87
Total	149	138	132	1205	1155	966	159	96

Table 4.2 Results for the GLMMs on proportion of extra-pair (EP) chicks in a male's social brood and total genetic offspring of a male, in relation to light treatment in his territory and distance to the nearest lamp post, for both 2013 and 2014 together and separate. The model output for the treatment with distance with year interaction term is given for the analysis of both years together. For the separate years, the model output is given for the treatment with distance interaction term, treatment and distance main effects (likelihood ratio test statistics are given for the step of the backward selection before the term was taken out), and, if the interaction was significant, the effect of distance in treatment subsets was tested. The sample size (n), chi-square test statistic (χ^2), degrees of freedom (d.f.) and significance level (p) are given for each term, significant p-values (<0.05) are underlined.

|		2013 & 2014					2013				2014			
		n	χ^2	d.f.	p		n	χ^2	d.f.	p	n	χ^2	d.f.	p
Proportion EP chicks per brood	Treatment : Distance : Year	149	9.00	3	0.03	Treatment : Distance	55	5.81	3	0.12	94	38.20	3	<0.001
						Treatment	55	2.12	3	0.55				
						Distance	55	0.45	1	0.50				
						Distance in dark	(14)				25	4.72	1	0.03
						Distance in green	(13)				22	1.36	1	0.24
						Distance in red	(16)				24	5.75	1	0.02
						Distance in white	(12)				23	16.57	1	<0.001
Total offspring per male	Treatment : Distance : Year	132	8.49	3	0.04	Treatment : Distance	47	4.56	3	0.21	85	5.41	3	0.14
						Treatment	47	0.99	3	0.80	85	0.89	3	0.83
						Distance	47	0.41	1	0.53	85	1.18	1	0.28

4.3 Results

The proportion of EP offspring in a brood was affected by the light treatment and the distance of the nest box to the nearest lamp post in 2014, but not in 2013 (Table 4.2). In 2013, we found no differences between treatments in the proportion of extra-pair young in broods (Figure 4.1A). In 2014, there was a significant interaction between treatment and distance to the nearest lamp post; the proportion of EP chicks decreased with distance to the nearest lamp post in the dark treatment, while it increased with distance in the red and white light treatments (Figure 4.1B). We found a significant effect of the interaction between light treatment, distance and year on the total number of offspring sired by a male (Table 4.2). However, in each separate year, treatment nor distance or their interaction affected the total number of offspring sired (Figure 4.1C & 4.1D).

4.4 Discussion

Our study reveals an effect of nocturnal illumination on the proportion of EP young in broods in one out of the two study years. In 2014, the proportion of EP young in broods increased with distance to the red and white lamps, while the proportion of EP young in broods in the dark control decreased with distance. We did not find an effect of distance to the light in the green transects. However, when we compared the proportion of EP young in broods only in the three illuminated treatments, the relation with distance did not differ between treatments (subset 2014 broods in green, red and white: treatment with distance interaction χ^2_2=4.55, p=0.10), indicating that the patterns in green light do not differ significantly from those in the white and red light.

Pairs nesting close to red and white light poles had a relatively low number of EP young in their broods. Egg fertilization rate in females is quite stable within species and between populations (Brommer et al. 2010), and no treatment effect is expected on fertilization rate. Thus, the lower EP proportions close to the light are most likely due to the fact that females breeding closer to the light posts were less likely to engage in EP copulations, rather than to a difference in egg fertilization rate. This effect of artificial light on female fidelity could originate from changes in the motivation of females to engage in extra-pair copulations. Through a possibly advanced onset of dawn song in illuminated areas (Kempenaers et al. 2010; Nordt and Klenke 2013; Da Silva et al. 2014), females close to the light sources may have perceived their mate to be of high quality compared to other males, and females mated to high-quality males are more faithful (Kempenaers et al. 1992). On the other hand, females far from the
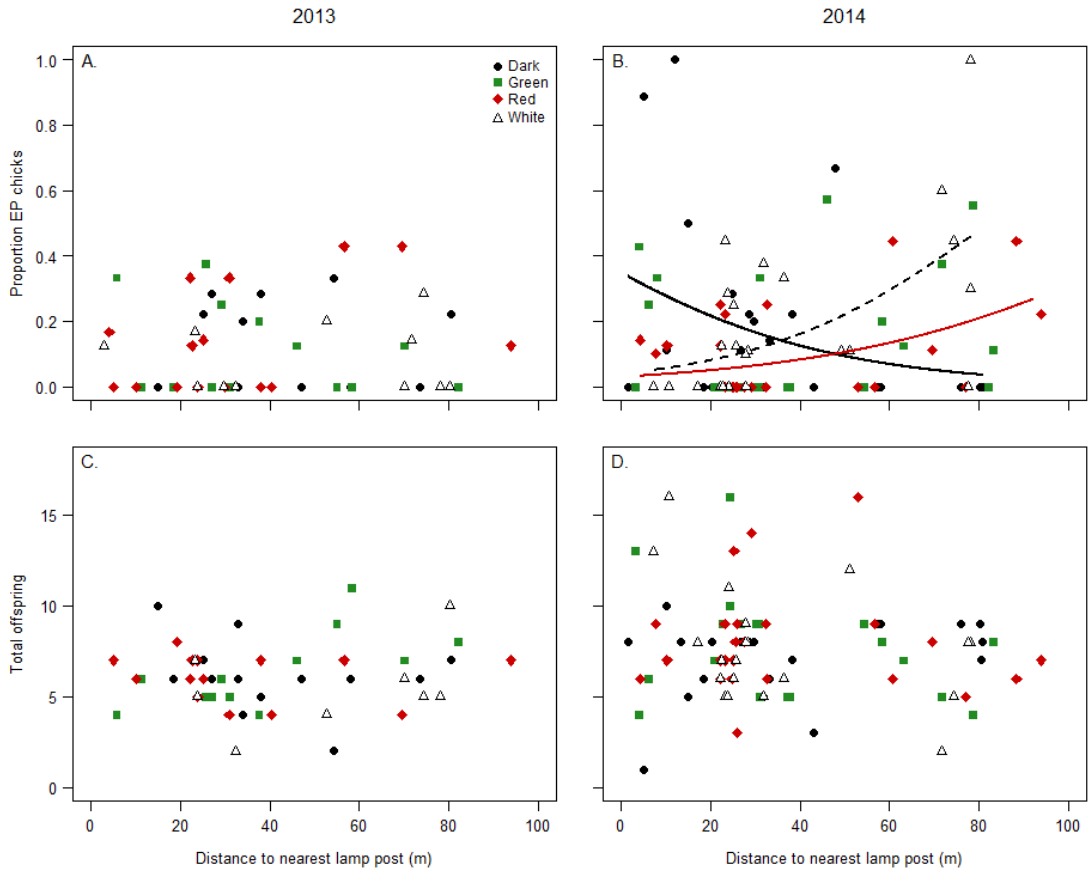
Figure 4.1 The proportion of extra-pair (EP) chicks in great tit broods, in relation to distance of the nest box to the nearest lamp post, in the four light treatments, for 2013 (A) and 2014 (B). The total number of genetic offspring of a male, in relation to distance of his social nest box to the nearest lamp post, in the four light treatments, for 2013 (C) and 2014 (D). Filled black circles are males in the dark treatment, filled green squares are males in the green treatment, filled red diamonds are males in the red treatment and open triangles are males in the white light treatment. Plotted lines in B are model predictions for treatments where distance significantly affected the proportion of EP chicks (solid black for dark treatment, solid red for red treatment and dashed black for white light treatment).

light sources might have perceived their mates to be of relatively low quality, and engaged more in extra-pair copulations. An alternative explanation is that light at night affects the mate guarding behaviour of males. Females spend the night in the nest box, where they are marginally affected or unaffected by the artificial light, whereas their social males may have experienced brighter conditions. A male

may be more successful in mate guarding via earlier onset of activity in lighted areas (Dominoni et al. 2014; de Jong et al. 2016).

In unlit control areas, we found that birds breeding close to the dark control poles had more EP young in their broods than those breeding farther away. This can be explained by the spatial pattern of our nest boxes at every transect, where the density of boxes decreases with increasing distance to the poles (de Jong et al. 2015). Great tits nesting close to the poles – and close to poles without lights at the dark control transects – potentially have the largest number of neighbours. Abundance of neighbours provides ample opportunities for females to take part in extra-pair copulations. Breeding density, both on a large and small scale, is a predictor of extra-pair paternity rate (Westneat and Sherman 1997; Stewart et al. 2010), which may explain the higher EP proportion close to the poles in the absence of light. We note that despite the same density effect in the illuminated areas, we find differences in EP offspring in the opposite direction as predicted by the density effect.

In 2013, we found no effect of nocturnal illumination on the proportion of EP young in broods, in contrast to 2014. We may not have had enough statistical power to detect an effect of light at night in 2013, due to the lower sample size in that year. Climatic conditions differed substantially between both breeding seasons, with 2013 being a very late and 2014 a very early spring (mean first egg laying date differed 20 days; de Jong et al. 2015). This difference is likely one of the reasons that sample sizes for 2014 were larger. In addition, in 2014, we used data from one more study area, which was not yet illuminated in 2013 and had a generally high great tit breeding density. However, excluding the 2014 data from this area did not change the results for proportion EP or total offspring. The decrease of EP young with distance to the dark control poles in 2014, as discussed above, was absent in 2013 (subset 2013 broods in the dark treatment: distance χ^2_1=0.40, p=0.53). The nest boxes in the dark with lowest distance from the poles were not occupied in 2013 (Figure 4.1A), which can explain the absence of the density effect.

Following from our hypotheses and the effect of light at night on proportion of EP chicks in broods, we would expect that males breeding in illuminated territories have more offspring in total, since they have less EP offspring in their own brood and may be more attractive for EP copulations with other females. However, the total number of offspring sired by a male, those in his own nest plus the ones as extra-pair in other nests, was not affected by artificial light at night. This demonstrates that the observed changes in fidelity of females breeding in illuminated territories in 2014 did not translate into substantial fitness benefits

for their males. This high sexual fidelity of females breeding in illuminated areas may have reduced the opportunities for males to gain paternity elsewhere. Numbers of offspring were not confounded by effects of light at night on brood size, because brood size (number of chicks that hatched) was not affected by light treatment or distance to the light (only year had a significant effect; average brood size in 2014 (8.0 chicks) was larger than in 2013 (6.9 chicks)). EP offspring in great tits have significantly shorter free-running periods (a measure of the length of the internal circadian rhythm) than WP offspring (Helm and Visser 2010). This, combined with the high heritability of period length (Helm and Visser 2010), suggests that EP fathers are active earlier. The lower proportion of EP offspring in males' social broods close to light may consequently result from enhanced mate guarding, rather than higher attractiveness of the social male, which would explain why the male's total number of offspring was unaffected by light at night. A recent study by Greives et al. (2015) provided wild great tit males with continuous night-time levels of melatonin, this delayed their daily onset of activity, and nestlings of these males were more likely to be sired by an EP male. These results strongly support our hypothesis that the lower EP offspring proportions observed in broods close to the light are due to an advanced onset of activity of the social male.

If an earlier onset of activity of the males by artificial light indeed causes males to be more successful at mate guarding, this could explain the difference with the findings of Kempenaers et al. (2010) on blue tits. There, no effects were found on the proportion of EP young (paternity loss) in broods in artificial light. In blue tits, there seems to be no relationship between unfaithfulness and emergence time (Schlicht et al. 2014) and thus artificial light would not be expected to aid mate guarding in blue tits. Whereas blue tit females exhibit strong sexual preferences in both their extra-pair and within-pair partner choice based on specific male characteristics (Kempenaers et al. 1997; Poesel et al. 2006), the importance of specific male characteristics that female great tits use for extra-pair mate selection is less clear (Strohbach et al. 1998; Kawano et al. 2009). This preference could explain why paternity gain in blue tits is so strongly affected by artificial light, probably by advancing the onset of dawn song (Kempenaers et al. 2010), whereas paternity gain is not for great tits.

One of the aspects of male quality is age. Many studies have shown a higher extra-pair siring success of older males (Kempenaers et al. 1997; Foerster et al. 2003; Poesel et al. 2006; Kempenaers et al. 2010). In our data, for both years, adding male age in the model for total offspring did not change the results. Also, there was no difference in the total number of offspring between 2nd calendar year males and males older than 2nd calendar year.

We are aware of the fact that individual birds were free to choose nest boxes at different distances to the lamp posts. Hence, a non-random selection of the population may breed in nest-boxes under light at night. However, we have shown in an earlier study on the same populations of birds (de Jong et al. 2015) that the breeding density did not differ between light treatments, and birds that survived from 2013 to 2014 did not move to a particular light colour or away from the illuminated area to the dark control. Although there is no reason to assume that males that were more attractive (and hence are mated to females that are less likely to engage in extra-pair copulations) settled in more illuminated territories, we cannot exclude this.

We show that the relation between the proportion of EP young and distance to the light in 2014 did not significantly differ between green, red and white treatments. However, the distance effect was specifically present in red and white light. This was also the case in a study on stress hormone concentrations in the same experimental set-up: adults nesting in white or close to red illumination had elevated corticosterone levels (Ouyang et al. 2015). Our red and white light both include larger proportions of longer wavelength radiation compared to our green light (Spoelstra et al. 2015). Long wavelength light is known to penetrate the skull more easily and has been found to be more effective at inducing a photoperiodic response (Hartwig and van Veen 1979), stimulating gonadal development and promoting body fattening than short wavelength light (Malik et al. 2002). Also, nocturnally migrating birds are disoriented by and attracted to white and red, but less to green and blue light (Wiltschko and Wiltschko 1995; Poot et al. 2008). Our results are consistent with this pattern and suggest that extra-pair paternity is more strongly affected by long wavelength than short wavelength light. More years of data are needed to get better insight in the extra-pair mating dynamics under artificial light at night, particularly in the role of light spectra.

We found that artificial light at night, in absence of other anthropogenic disturbance, potentially disrupts the natural patterns of extra-pair paternity. This disruption could lead to maladaptive mate choice decisions of females (Kempenaers et al. 2010). Great tits breeding at experimentally illuminated transects in natural habitat showed a reduced proportion of EP young in one of the two study years. Potential benefits of extra-pair copulations (Foerster et al. 2003) may therefore be negated by nocturnal illumination. Our finding that there are fewer EP offspring in illuminated broods thus shows that light at night disrupts sexual selection processes in wild birds.

Funding

Acknowledgements

Chapter 5

Do wild great tits avoid exposure to light at night?

Maaike de Jong
Jenny Q. Ouyang
Roy H.A. van Grunsven
Marcel E. Visser
Kamiel Spoelstra

Abstract

Studies of wild populations have provided important insights in the effects of artificial light at night on organisms, populations and ecosystems. However, in most studies the exact amount of light at night individuals are exposed to remains unknown. Individuals can potentially control their night-time light exposure by seeking dark spots within illuminated areas. This uncertainty makes it difficult to attribute effects to a direct effect of light at night, or to indirect effects, for example, via an effect of light at night on food availability. In this study, we aim to quantify the nocturnal light exposure of wild birds in a previously dark forest-edge habitat, experimentally illuminated with three different colours of street lighting, in comparison to a dark control. During two consecutive breeding seasons, we deployed male great tits (*Parus major*) with a light logger measuring light intensity every five minutes over a 24 h period. We found that the males from pairs breeding in brightly illuminated nest boxes close to lamp posts, were not exposed to more artificial light at night than males from pairs breeding further away. This suggests that these males could have been actively avoiding light at night by choosing a roosting place with a reduced light intensity. Therefore, our findings suggest that effects of light at night previously reported for this species in our experimental set-up are potentially indirect. In contrast to urban areas where light is omnipresent, bird species in non-urban areas may actively evade exposure to nocturnal artificial light, thereby avoiding direct consequences of light at night.

5.1 Introduction

Natural night-time darkness has disappeared across large parts of the world (Cinzano et al. 2001) as a result of anthropogenic lighting of the environment. Effects of artificial light at night on ecosystems are increasingly being studied over the past decade (Rich and Longcore 2006). In order to assess latent and ecosystem-wide consequences, long-term experiments have been set up, for example, in Germany (Hölker et al. 2015), the United Kingdom (Bennie et al. 2015) and the Netherlands (Spoelstra et al. 2015). These are starting to provide important insights in impacts of light at night on plant and animal populations, such as the suppression of flowering (Bennie et al. 2015), the alteration of microbial communities (Hölker et al. 2015) and the suppression or facilitation of mammal activity (Spoelstra et al. 2015).

Effects of artificial light at night on an individual can be both direct and indirect. For example, a change in activity pattern could be a direct effect: the individual is exposed to the light, which affects its physiology and behaviour (e.g., timing of reproductive physiology in birds; Dominoni et al. 2013a). But the same effect can also be indirect: light at night attracts prey species for the focal individual which consequently may change foraging activity (e.g., bats feeding on moths near lamps; Wakefield et al. 2015).

Optimally, light intensities in experiments are chosen such that they are comparable to 'real-life' outdoor lighting situations. The light levels around light sources can be precisely measured but often the focal species is highly mobile. It is therefore difficult to know to how much light the studied individuals are actually exposed to, as these individuals are well able to move away from the light. Consequently, it becomes difficult to relate effects to experienced light levels, and effects observed in individuals that succeed to evade light at night may rather be indirect.

Few studies so far have measured nocturnal light levels as experienced by individual free-living animals. Dominoni et al. fitted rural and city blackbirds (*Turdus merula*) with light loggers and related individual light exposure to timing of daily activity (Dominoni et al. 2014) and subjective perception of day length (Dominoni and Partecke 2015). Robert et al. (2015) linked melatonin levels and timing of seasonal reproduction to exposure of individual tammar wallabies (*Macropus eugenii*) to light at night. Although in these cases the night-time light exposure of focal individuals is known, these studies remain correlative since other anthropogenic factors that typically co-occur with artificial light, such as noise, cannot be excluded.

In an experimental set-up, designed to assess the effects of artificial light at night of different colours on wild birds, we have so far observed that light at night can advance timing of egg laying (de Jong et al. 2015) and increase corticosterone levels in the great tit (*Parus major*; Ouyang et al. 2015). It remains unclear whether these effects directly relate to increased light intensities at night, or whether these effects are more indirect. Information on the actual light exposure of individuals is essential in understanding its effects. We know precisely how the light intensity varies with distance to the lamps in this set-up, but we do not know whether birds avoid exposure to nocturnal artificial light as they can move away from the lamp posts at night and roost overnight in much darker places. Here, we assess to how much light individual great tits are exposed at night, and relate this to light levels at the location of their nest box.

5.2 Methods

5.2.1 Study area
We made use of a field site, Voorstonden, which is part of a long-term experiment in the Netherlands. In this experiment, previously dark natural areas are illuminated with white, green and red light, in comparison to a dark control area. For details about the set-up of this experiment and the characteristics of the light, see Spoelstra et al. (2015). The field site of this study is situated east of the Veluwe area (52°7'21'' N; 6°7'7'' E) and consists of deciduous and mixed forest edge habitat, with few natural cavities, and semi-natural grassland. Perpendicular to the forest edge, four transects have been set up, each with five lamp posts. Each transect contains one of the four light treatments (white, green or red LED light, or dark control), and nine bird nest boxes at approximately 25 m distance from each other in a grid around the lamp posts. For details about the study on nest box breeding birds and a schematic overview of the field site, see de Jong et al. (2015). Light intensity at all nest box entrances was measured in four directions (upward, forward, to the left and to the right) with a calibrated illuminance meter (LMT B 360, LMT Lichtmesstechnik GmbH, Berlin, Germany). Averages of these four measurements are presented in Appendix Figure 5.A1 for the nest boxes located within 30 m distance of the nearest lamp post.

5.2.2 Light logger measurements
We measured the light intensities that free ranging male great tits are exposed to at night with miniature light loggers (custom-made by Sigma Delta Technologies, Floreat, Western Australia, Australia) with a weight of ~0.95 g. including harness. The light sensor (ISL29033, Intersil, USA) has a measuring range of 0.055 to 125 lux and a spectral sensitivity range from 300 to 700 nm. The sampling interval was set to five minutes and loggers were active for at least 24 hours. In 2014,

between April 24 and May 16, and in 2015, between May 11 and 26, in total 30 birds were equipped with a light logger. We caught the males of great tit pairs that were nesting in a nest box within 30 m of a lamp post or a dark control pole. During the second half of the egg incubation phase (eight nests in 2014, none in 2015), males were caught close to their nest box using a mist net and song play-back. During chick feeding phase (seven nests in 2014, 15 nests in 2015), males were caught in the nest box using a spring trap. Birds were ringed with a numbered aluminium ring and the light logger was attached to their back using a leg loop harness (photograph of bird with logger in Appendix Figure 5.A2). We aimed to evenly distribute the loggers over the four light treatments, but were dependent on the presence of great tit nests (see Table 5.1 for number of deployments in each treatment). To retrieve the loggers and to collect the data, we tried to recapture birds during the same breeding season, using a spring trap or a mist net close to their nest box.

5.2.3 Light logger effects on nestling survival

When designing our light loggers, mass was the primary limiting factor. Male great tits weigh 18-19 g during the breeding season, which means that a light logger of ~0.95 g adds about 5% to their body weight. Although it is widely accepted that devices that add a maximum of 5% to the body mass of a bird do not significantly affect its behaviour (Aldridge and Brigham 1988), a recent meta-analysis (Barron et al. 2010) showed that attaching devices to birds in general negatively affects most aspects of their behaviour and ecology. To test whether deploying light loggers had a negative effect on parental care, we compared nestling survival (number of chicks that fledged / number of chicks that hatched; 1 = all chicks that hatched successfully fledged and 0 = no chicks that hatched successfully fledged), of the nests of which the male received a light logger (n=14 in 2014, n=15 in 2015) to those of which the male did not receive a light logger (n=7 in 2014, n=9 in 2015). All great tit nests at the field site were inspected regularly to assess the number of chicks that hatched and the number of chicks that fledged. We used a Mann-Whitney U test to compare nestling survival between nests with and without logger in each year, and found no differences (nestling survival in 2014: 0.93 ± 0.04 with logger and 0.95 ± 0.05 without logger (avg ± s.e.), Mann-Whitney U test: W=55, p=0.58; and in 2015: 0.82 ± 0.08 with and 0.78 ± 0.11 without logger (avg ± s.e.), Mann-Whitney U test: W=57.5, p=0.54). Nestling survival was generally high in this area and we found no difference between pairs with and without light logger, thus we assume that the loggers did not cause behavioural differences that would affect reproductive success between the two groups of males.

Table 5.1 Number of male great tits that were deployed with a logger, were caught back, and for which data are available in 2014 and 2015 for the separate treatments and the totals. See also Appendix Figure 5.A4 for the breeding locations of the light logger males.

		Dark	Green	Red	White	Total
2014	Birds deployed	4	3	4	4	15
	Birds caught back	2	3	4	3	12
	Data available	0	2	2	2	6
2015	Birds deployed	6	2	3	4	15
	Birds caught back	2	1	3	2	8
	Data available	1	1	3	2	7

5.2.4 Data analysis

We validated the readings of the light loggers with a calibrated illuminance meter (LMT B 360) for all three light colours (see for details Appendix Figure 5.A3) and the original light logger measurements collected on birds were corrected for deviations from the illuminance meter measurements. We limited data analysis to the first 24 hours (for which we have data from all birds) and calculated the average light intensity received by the birds between two hours after sunset and two hours before sunrise (on average 4.5 hours). We excluded the hours after sunset and before sunrise, because earlier studies have shown that daily activity patterns are specifically affected by artificial light at night during these periods (Dominoni et al. 2013b; de Jong et al. 2016). This way the measurements pertain to the resting period and are not confounded by shifts in activity patterns. We related the log of light intensity as measured on the bird, to the log of light intensity at the entrance of the nest box the pair was breeding in with a Spearman's rank correlation test. Also, for the illuminated transects, we related both the logger and the nest box entrance average log light intensity to the distance between the nest box and the nearest lamp post, again using a Spearman's rank correlation test.

Ethics statement

Natuurmonumenten granted us permission to perform our experiment on their terrain; the natural area Voorstonden. The study was approved by the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences and carried out under licence NIOO 10.07.

5.3 Results

We were able to recapture 20 out of the 30 male great tits that were deployed with loggers, and we obtained light intensity data from 13 of them (see Table 5.1; in 2014 we obtained data from three loggers during egg incubation phase and three during chick feeding phase, in 2015 we obtained data from seven loggers during chick feeding). Seven birds either lost their light logger or their logger failed

to record any data. In Appendix Figure 5.A4, we show where the 13 males, from which we obtained data, have been breeding.

The light intensity as recorded at the back of the male great tits did not change with increasing light intensity at the entrance of the nest box the pair was breeding in (Spearman's rank correlation test: rho=0.15, p=0.63; and see Figure 5.1). For the males of pairs breeding in nest boxes closer than 10 m to the light posts, the average light intensity at the entrance of nest boxes is about 100 times higher than the average light level measured at the birds (respectively 6.79 lux and 0.062 lux; Figure 5.1). A correlation test for light intensity and distance to the nearest lamp post showed that light intensities at nest box entrance (in the illuminated transects) significantly decreased with distance to the nearest lamp post (Spearman's rank; rho=-0.76, p<0.001, Appendix Figure 5.A1). The same test for the male great tits nesting in the illuminated transects did not show a correlation between received light intensity and distance to the nearest lamp post (rho=0.26, p=0.41).

5.4 Discussion

The light levels experienced by the male great tits nesting in the direct surroundings of the lamp posts are much lower than the light levels measured there. The reduced exposure to light is only possible when these three males do not roost in close vicinity of their nests (Figure 5.1). This suggests that these males have been actively avoiding light exposure at night by choosing a roosting place with a reduced light intensity, behind, or higher up in a tree, or further away from their nest box. It is unlikely that the low values measured on birds resulted from males roosting in another nest box because >95% of all nest boxes were occupied by breeding pairs of great tits or other species. Males' night roosting locations were within 10 m of their nest box location (Ouyang et al. in prep.). Males from pairs breeding close to the lamp posts were not exposed to more light than males from pairs nesting further away, thus the breeding pair's choice of nest location, in this area, does not influence nocturnal light exposure. Unfortunately, we had only one measurement of a male in the dark control treatment, but the fact that this male was exposed to a light intensity not different from those in the illuminated treatments (Figure 5.1) supports our conclusion. The possibility of birds avoiding light exposure at night was already touched upon by Dominoni et al. (Dominoni et al. 2014); although the urban blackbirds in their study were exposed to higher light intensity at night than rural conspecifics, this intensity was at least 20-fold lower than the light intensity measurable in a 30 m radius from a common street lamp in the urban sites.
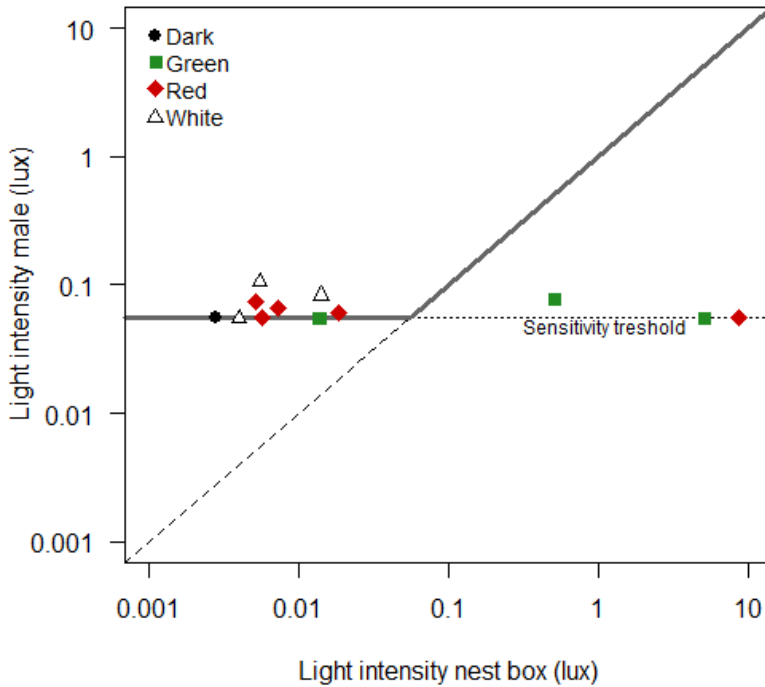
Figure 5.1 Average nocturnal light intensity (lux) that male great tits were exposed to (from two hours after sunset to two hours before sunrise), in relation to the light intensity (lux) measured at the nest box the pair was breeding in. The grey line indicates the expected light exposure if males would roost in the close surroundings of their nest box; up to 0.055 lux this remains level because of the lower sensitivity threshold of the loggers (dotted line) and at higher intensities this is equal to light intensity measured at nest box entrance level (dashed line: light intensity male equals light intensity nest box). Filled black circles are males with nest in the dark treatment, filled green squares are males with nest in the green treatment, filled red diamonds are males with nest in the red and open triangles are males in the white light treatment. Data from breeding seasons 2014 and 2015; error bars are not shown because standard errors are too small to be visible. Original light logger measurements were corrected to be comparable with the illuminance meter measurements in Appendix Figure 5.A1, see logger calibration data in Appendix Figure 5.A3.

Although we have shown that nestling survival in nests of males that were deployed with a light logger was not lower than in other nests, we cannot exclude that initial stress by capture and restraint has influenced the behaviour of the males (Calvo and Furness 1992; Murray and Fuller 2000). However, for birds from which we obtained more than 24 hours of data, light levels in the second night did

not differ from those measured in the first night. Therefore, it seems unlikely that the observed avoidance of light during the first night results from an initial stress response.

The elevated corticosterone levels previously found in great tits nesting in the illuminated areas in this project could be a direct consequence of the light at night in the form of sleep disturbance (Raap et al. 2015), restlessness or alterations in circadian rhythms. Alternatively, these elevated levels could indirectly result from increased metabolism due to increased food availability and/or feeding rates, as discussed in Ouyang et al. (2015). The data we present here imply that the reported physiological changes may well be an indirect effect. Similarly, the advancement in lay date of great tits, as discussed in de Jong et al. (2015), could be directly caused by a changed perception of day length, or, more likely in the light of the data presented here, could be related to a change in (timing of) abundance of prey species as a result of the artificial light at night.
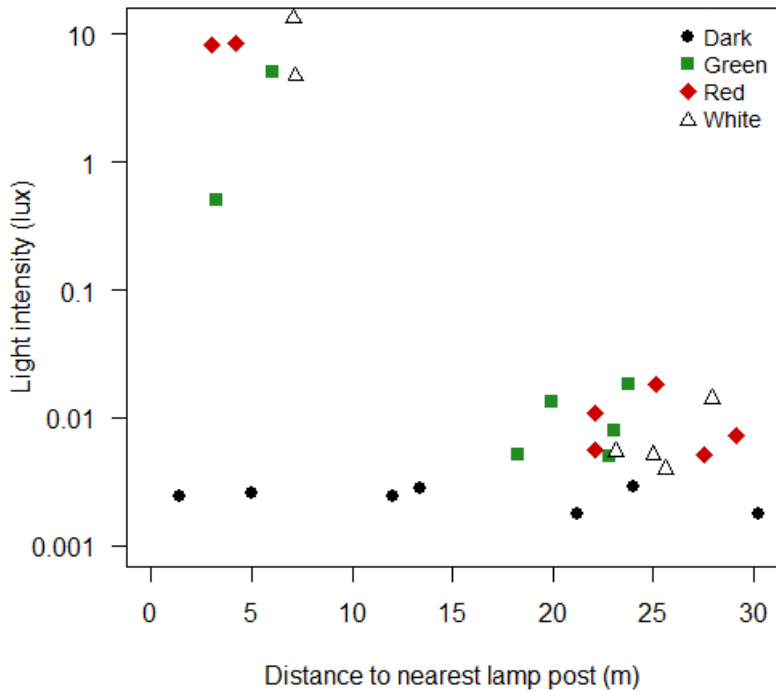
The lower sensitivity boundary of our light loggers is 0.055 lux. This allows us to compare light levels in the direct surroundings of the lamp posts with levels that occur further away from them. However, light levels around 25 m distance from the lamps cannot be distinguished from background light levels as measured in the dark transects (see Appendix Figure 5.A1). Our measurements of light levels are done at only one field site, and the sample size is relatively low; we were able to obtain data from 13 males. The findings presented here, suggest the presence of indirect pathways of effects of nocturnal illumination, but more measurements are needed for a conclusive statement of how different light spectra affect behaviour in free-living songbirds.

In rural and (semi-)natural areas, such as our study area, illumination is most often a linear structure like lighting along a road, with ample dark places around where birds can escape direct light exposure. In our experimental set-up, birds can use this possibility; male great tits seem to actively avoid artificial light at night. In this perspective, rural and (semi-) natural areas differ fundamentally from urban areas where light levels are not only higher but dark places needed to avoid exposure are less easy to find. The blackbirds in urban areas studied by Dominoni et al. are exposed to a generally higher light intensity, but also a higher variability, compared to rural birds (Dominoni et al. 2014). Likewise, Robert et al. show that wallabies experience orders of magnitude more light at night in an urban compared to a natural area (Robert et al. 2015). Differences in habitat structure and availability of dark areas between urban and non-urban environments thus result in different nocturnal exposure of birds and effects demonstrated in urban areas may not be easily extrapolated to more natural areas.

Acknowledgements

Appendix

Figure 5.A1 Light intensity (lux) at nest box entrance level in relation to distance to the nearest lamp post (m) for all four light treatments; filled black circles are nest boxes in the dark treatment, filled green squares are nest boxes in the green treatment, filled red diamonds are nest boxes in the red and open triangles are nest boxes in the white light treatment. We present the average light intensity value of measurements, done with a calibrated illuminance meter, LMT B 360 (LMT Lichtmesstechnik GmbH, Berlin, Germany), in four directions (upward, forward, to the left and to the right) at each nest box entrance within 30 m distance of the nearest lamp post.

Figure 5.A2 Male great tit deployed with light logger 'E' (written on the round battery). The logger is attached at the back of the bird with a leg loop harness. The size of the leg loops was adjusted to match the bird during deployment. The light sensor is located just underneath the battery (indicated with red in the enlargement), such that normally, within a few days, it should not be covered by feathers. Photograph made by J.Q. Ouyang on 24/04/2014 at field site Voorstonden, just before release of the bird with light logger.
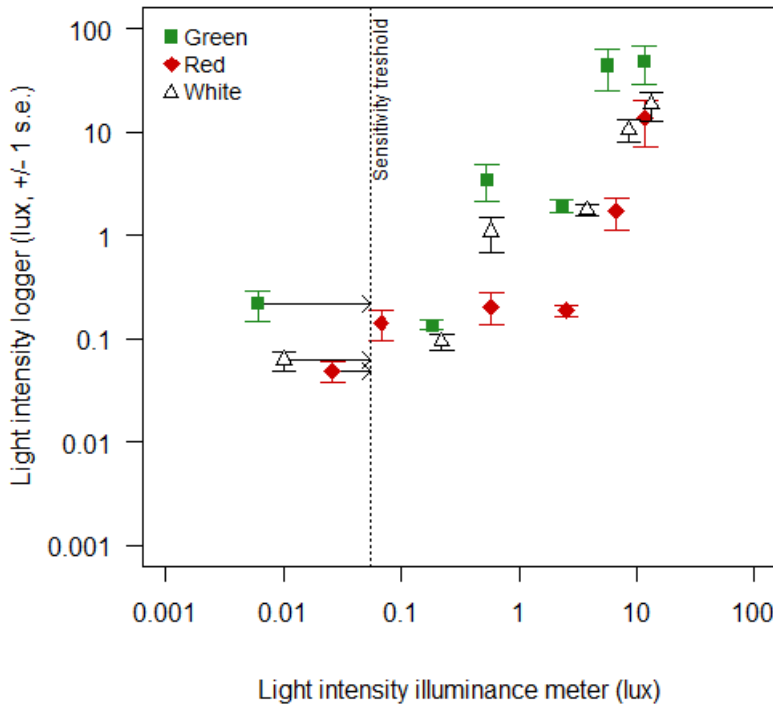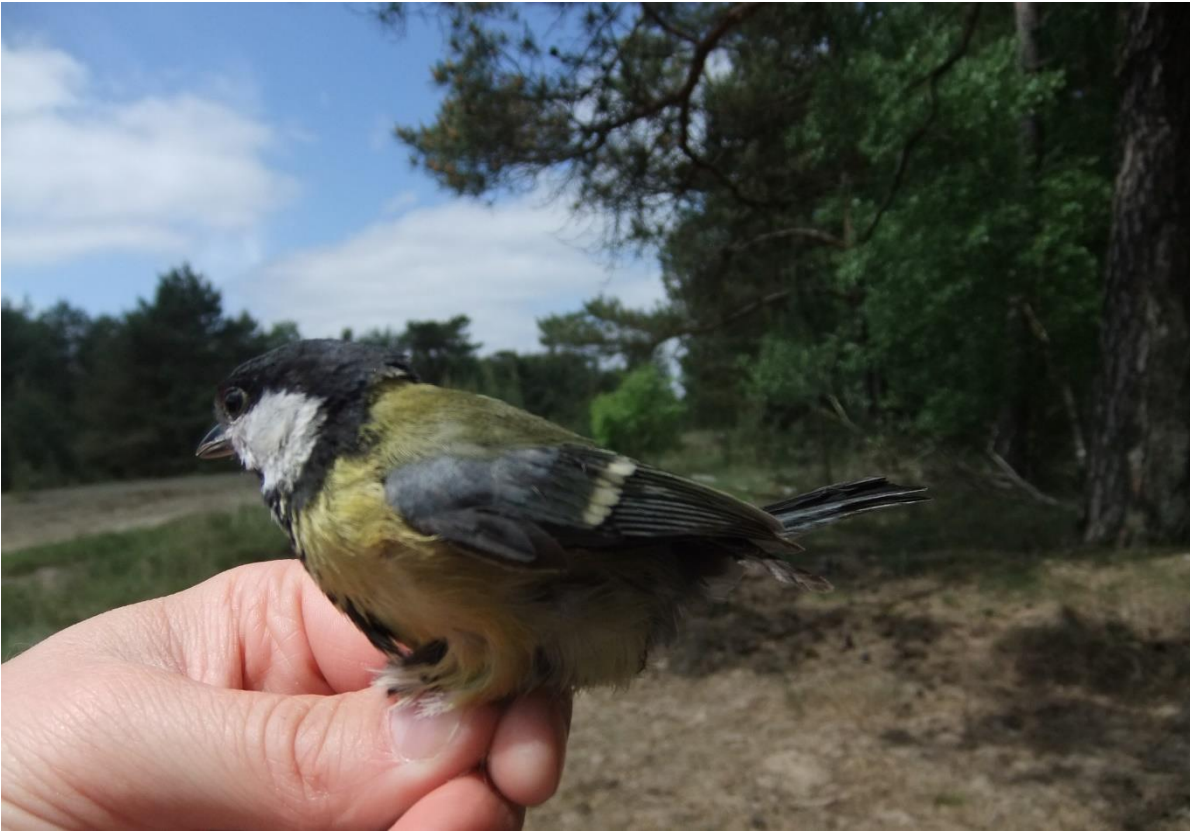
Figure 5.A3 The validation of the readings of the light loggers was done indoors, in a completely dark room, for each of the three light spectra (filled green squares for measurements under green light, filled red diamonds for red light and open triangles for measurements under white light). Measurements were done with a calibrated illuminance meter, LMT B 360 (LMT Lichtmesstechnik GmbH, Berlin, Germany), and at the same location with four light loggers, in three different directions (light sensor of logger facing towards the light source, away from the light source and horizontally with 90° angle to the light source). The averages of the light intensity in the three directions, measured by four light loggers (lux, ± 1 s.e.), in relation to the measurements of the illuminance meter (lux) are plotted. The sensitivity threshold of the light loggers is 0.055 lux (indicated with dotted line), which means that all measurements below this threshold are not reliable and are set to 0.055 lux (indicated with black arrows).

Figure 5.A4 Schematic overview of our study site, Voorstonden. Five lamp posts (here green, white and red light respectively) and five dark control poles are placed in transects perpendicular to the forest edge. In each transect, nine nest boxes were attached to trees at 1.6 m height and at approximately 25 m distance from each other (dependent on the nearest tree). Orientation of the nest box opening was always towards the forest edge. Yellow squares indicate where the six males with light logger from which we obtained data in 2014 have been breeding. Yellow circles indicate where the seven males with light logger from which we obtained data in 2015 have been breeding. Note the nest box in the white transect from which we have data in 2014 as well as in 2015. Figure adapted from: de Jong M, Ouyang JQ, Da Silva A, van Grunsven RHA, Kempenaers B, Visser ME, Spoelstra K. Effects of nocturnal illumination on life-history decisions and fitness in two wild songbird species. Philosophical Trans R Soc B Biol Sci. 2015;370:20140128.

Chapter 6

Dose-dependent responses of avian daily rhythms to artificial light at night

Maaike de Jong
Lizanne Jeninga
Jenny Q. Ouyang
Kees van Oers
Kamiel Spoelstra
Marcel E. Visser

Abstract

Recent studies have shown that animals are affected by night-time light exposure. Light is a continuous variable, but our knowledge on how individuals react to different light intensities during the night is limited. We therefore determined the relationship between night light intensity and the behaviour and physiology of great tits (*Parus major*). We measured daily activity patterns and melatonin levels in 35 males exposed to five different light intensities and found strong, dose-dependent effects. Activity onset was increasingly advanced, and activity offset delayed with higher light intensities. Furthermore, night-time activity increased and melatonin levels measured at midnight decreased with higher intensities. In this experimental study, we demonstrate for the first time dose-dependent effects of artificial light at night on birds' daily activity patterns and melatonin levels. Our results imply that these effects are not limited to a certain threshold, but emerge even when nocturnal light levels are slightly increased. However, in a natural area, these effects may be limited as artificial light levels are commonly low; light intensities drop rapidly with distance to a light source and birds can avoid exposure to light at night. Future studies should thus focus on examining the impact of different intensities of light at night in the wild.

6.1 Introduction

Research recently started to focus on the dramatically changed night-time light conditions, and our understanding of the ecological consequences of light pollution is still limited (Rich and Longcore 2006; Gaston et al. 2013; Spoelstra et al. 2015). Artificial lighting of urban and rural areas will continue to increase worldwide (Cinzano et al. 2001) and can have major effects on the behaviour and fitness of wild species (Hölker et al. 2010). Besides very direct and often lethal effects, such as the well-known fatal attraction of sea turtle nestlings to coastal lights (Kamrowski et al. 2014) and avian mortality from collisions with human-made illuminated structures (Longcore et al. 2013), more subtle effects can occur due to the disruption of natural daily cycles of light and darkness as well as seasonal cycles in day length that are used to anticipate environmental changes (Bennie et al. 2014).

In birds, internal circadian and circannual clocks are synchronized by light stimulation of photoreceptors (Dawson et al. 2001). This photosensitivity enables birds to align their activity and physiology to the appropriate time of the day and year. Recent studies on wild populations show that blackbirds (*Turdus merula*) in more light polluted areas perceive a longer subjective day than conspecifics in darker regions (Dominoni and Partecke 2015) and that they extend foraging activity into illuminated nights (Russ et al. 2015). Great tits (*Parus major*) that were exposed to light inside their nest box woke up and left their nest box earlier in the morning (Raap et al. 2015). Also, timing of dawn and dusk singing in common songbirds was altered by light at night (Da Silva et al. 2015). In addition, multiple studies showed an effect of artificial light on timing of reproduction, such as in the blue tit (*Cyanistes caeruleus*; Kempenaers et al. 2010), the blackbird (Dominoni et al. 2013a) and the great tit (de Jong et al. 2015).

The presence of light at night cannot be indicated as a 'yes or no' event, but is a disturbance of natural habitat which continues from bright light close to the light source to very low light intensities at greater distance. Knowing the behavioural response to different light intensities and consequent effects on reproduction and survival is therefore necessary to quantify the impact of artificial light on bird populations. Gaston et al. (2015) recently concluded that so far most studies have focused on light at night versus no light at night, and that one of the important research challenges is to determine the thresholds and dose-response functions for biological impacts of artificial light at night. One of the few examples of this kind of studies is on the dispersal of Atlantic salmon fry (*Salmo salar*; Riley et al. 2015), in which researchers identified the intensity at which artificial light disrupted dispersal behaviour: the threshold for delaying dispersal was reached

at low light intensity, with little additive effect of increasing light intensity up to eight lux. Altered daily activity patterns have previously been found in the Indian weaver bird (*Ploceus philippinus*), where activity in the subjective night increased with higher light levels (Singh et al. 2012), although daytime light levels in this study were relatively low.

The hormone melatonin plays an important role in the circadian organisation of birds and other vertebrates. Melatonin is released by the pineal gland during the dark phase of the day and suppressed by (day-) light via photoreceptors (Bell-Pedersen et al. 2005; Cassone 2014). It accurately encodes the duration of the night and hence day length, thereby helping birds to synchronise their behaviour and physiology to the external light-dark cycle (Gwinner et al. 1997). Melatonin is known to be related to locomotor activity and both are regularly measured in relation to effects of light at night (Dominoni et al. 2013b; Yadav et al. 2015). We expect artificial light at night to suppress melatonin levels, which was recently shown to be the case in the tammar wallaby (*Macropus eugenii*; Robert et al. 2015), in the Indian weaver bird (Singh et al. 2012) and in the blackbird (Dominoni et al. 2013b). In fish, circadian melatonin patterns were inhibited by low intensity night light levels (Brüning et al. 2015). In contrast, a study on western scrub-jays (*Aphelocoma californica*) showed opposite effects of light at night: amplitude of melatonin was increased (Schoech et al. 2013). Therefore, it is still largely unknown how activity patterns and melatonin levels relate to intensity of light at night.

In our study, we determined the dose-response relationship for the effect of night light intensity on the daily rhythms of a small passerine, the great tit. In a laboratory setting, we exposed birds to five night light treatments, varying from 0.05 to 5 lux. These intensities are comparable to light levels around lamp posts in rural areas in most of Northern Europe (Commissie Openbare Verlichting 2011), and those on the lower end have been empirically measured on European blackbirds that carried light loggers (Dominoni et al. 2014). We continuously measured daily activity patterns. We sampled plasma melatonin levels at midday to obtain baseline levels, at midnight to determine suppressive effects of light, and shortly before morning light to measure possible changes in melatonin timing reported in blackbirds (Dominoni et al. 2013b). We hypothesize that daily activity patterns are altered under light at night and that the effects are larger with increasing light intensity. We expect secretion of melatonin to be progressively depressed at midnight with increasing intensities of light at night. To test for possible carry-over effects, we used a longitudinal design, in which we exposed each bird first to dark nights, then to artificial light at night, and then again to dark nights. In addition to information on carry-over effects, this design also accounts

for possible changes in the circadian system as the season progressed (Daan and Aschoff 1975). We expect no differences between the treatments for behaviour as well as physiology when comparing the first and last period, without nocturnal illumination.

6.2 Methods

6.2.1 Animals

For this experiment we studied 35 male great tits. Birds were hand raised and housed at the Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, the Netherlands. They were between one and four years of age, and had known pedigrees. Birds were housed in individual cages (90cm x 50cm x 40cm). Each cage had two separate light sources for day- and night-time illumination, and external light was completely excluded. Cages were ventilated and temperature was maintained between 10 and 14°C, and did not vary structurally between day- and night-time. Birds had access to food and water *ad libitum*. During the experiment, which lasted from 26 November 2013 to 31 January 2014, birds were kept on constant 8:15 hours light – 15:45 hours 'dark', which was the average natural photoperiod at the time the experiment took place. All experimental procedures were carried out under licence NIOO 13.11 of the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences.

6.2.2 Experimental set-up

The light treatment varied during three periods. During the first experimental period (26 November 2013 – 10 December 2013), birds were not exposed to light at night. In the second experimental period (10 December 2013 – 10 January 2014), birds were exposed to one out of five nocturnal light intensities, measured at perch level in the cages: 0.05 lux, 0.15 lux, 0.5 lux, 1.5 lux or 5 lux warm white LED light (Philips, Eindhoven, the Netherlands). For spectral composition see Appendix Figure 6.A1 and for exact intensity of each night light lamp see Appendix Figure 6.A2. During the third experimental period (10 January 2014 – 31 January 2014), birds were not exposed to light at night, as in the first period. The 35 individuals were assigned randomly to a treatment group and to one of seven blocks, each block containing all five night light treatments. These blocks were divided over two identical rooms. Treatments were allocated to cages using a Latin Squares design. Birds from the same family or age were distributed evenly across treatment groups. During daytime, 8:30h – 16:45h, birds were exposed to full spectrum daylight high frequency fluorescent lights emitting +/- 1000 lux at perch level (Activa 172, Philips, Eindhoven, the Netherlands). During experimental period 2, night light LED lamps were switched on at 16:30h and off at 8:45h, with

15 minutes overlap with the daylight lamps. One bird (treatment group 0.05 lux) died during the second experimental period due to an unknown cause.

6.2.3 Activity measurements

Daily activity patterns of each individual bird were measured continuously throughout the three experimental periods. Each cage was equipped with one normal wooden perch and one wooden perch fitted with a microswitch. This microswitch registered perch-hopping activity which was recorded on a computer (method used in Gänshirt et al. 1984; Astheimer et al. 1992). White noise was played continuously on a low level during day and night to mask the sound of the active perches and vocal activities of the birds. A zero (no connection, bird not on perch) – one (connection, bird on perch) signal was registered every 0.1 second and software developed by T&M Automation (Leidschendam, the Netherlands) created log files for each 30 second interval. Depending on whether birds were changing perches within each 30 second interval, we obtained four levels of activity per two minutes. In our activity analysis, we excluded the first five days of each experimental period, because birds had to acclimatize to their new environment or change of treatment. We also excluded the days that blood samples were taken. ChronoShop 1.1 (written by KS) was used to calculate four activity descriptors for each individual, in each 24 hour cycle (from midnight to midnight). The onset of activity is defined as the first moment the activity exceeds the average activity of the day. In order to avoid premature onsets, a running mean of 20 minutes length was fitted to the data. Activity onset is reported in minutes relative to the moment the daylight was switched on, 'start of day' (activity onset minus light on). The offset of activity, the last moment the activity was above the average activity value of the day, was calculated in the same way, also with a running mean of 20 minutes, and reported as minutes relative to the moment daylight was switched off, 'end of day' (activity offset minus light off). Total activity is defined as the total amount of active minutes (minutes in which the bird has been active) in a 24 hour cycle. Finally, nocturnal activity is calculated as the proportion of total activity that took place during the objective night (daylight off to daylight on).

6.2.4 Melatonin measurements

In order to determine plasma melatonin levels, we took nine blood samples from each bird over the course of the experiment. On the first day of the last week of each experimental period a sample was taken at midday (between 12:30h and 13:30h). Three days later, a sample was taken in the early morning, before daylight was switched on (between 06:30h and 07:30h). Another three days later, a sample was taken at midnight (between 00:00h and 01:00h). The catching order of the birds was randomized. At midnight and early morning sampling, birds

were caught from their cages and sampled under dim white light. Time between start of catching and blood sampling was 5:49 ± 0:10 minutes (mean ± s.e.). The wing vein was punctured and a sample of 70 µl was drawn and put on ice immediately. Directly after sampling all individuals, which took 55 – 91 minutes, blood samples were centrifuged and plasma was separated and stored at -80ºC. Plasma concentrations of melatonin were analysed at the Animal Endocrinology Laboratory (University of Ferrara, Italy) using a commercially available Multispecies 125-I Melatonin Research kit (Labor Diagnostika Nord GmbH & Co. KG, Nordhorn, Germany), see also Greives et al. (2012). Plasma samples were extracted with Dichlormethane then re-suspended in phosphate-buffered saline before proceeding with the radioimmunoassay (RIA). Samples were divided in two assays, with all samples from the same individual run in the same assay. Standard curve and data were calculated with ImmunoFit EIA/RIA Analysis (Beckmann Instruments). The lower detection limit corrected for volume was 42 pg/ml. Intra-assay coefficients of variation were 3.64% and 3.20% respectively and inter-assay coefficient of variation was 1.24%. Melatonin concentrations were adjusted for recoveries of 60%.

6.2.5 Statistical methods

All statistical analyses were done using R v. 3.1.2 (R Development Core Team 2014) with a significance level of α = 0.05. Initial models for the response variables were linear-mixed-effects models (LMM) with the interaction between treatment (a five level factor; 0.05 lux, 0.15 lux, 0.5 lux, 1.5 lux and 5 lux) and experimental period (a three level factor; experimental period 1, 2 and 3) as fixed effects and individual nested in block, which is nested in room as a random effect to account for repeated measures of the same individuals and possible effects of location of the cage. If the interaction between treatment and experimental period was significant, we performed a post-hoc analysis where we evaluated the effect of the five light intensity treatments in each of the three experimental periods, using the R package *phia*. For model comparisons an *F*-test was calculated according to the approach of Kenward and Roger (Halekoh and Højsgaard 2014). Nocturnal activity (proportion of total activity that took place during the objective night) was arcsine transformed before analysis. Assumptions for using linear models were met. Within experimental period 2, we tested whether our data was ordered as expected, following the order of the light intensity treatment, using the ordered heterogeneity test (OH test; Rice and Gaines 1994). Covariates age and family were one by one added in the models for onset, offset, total and nocturnal activity and were all not significant, except for age in the total activity model (1 year old birds: 337 ± 20.0 minutes; 2 years old: 266 ± 18.1 minutes; 4 years old: 344 ± 24.1 minutes (mean ± s.e.)). Covariates age, family and time between catch and sample were one by one added in the models for midday, midnight and

morning melatonin and none of them were significant. Significant covariates were kept in the models.

6.3 Results

6.3.1 Activity patterns

Actograms of five representative individuals, one from each treatment group, are shown in Figure 6.1. All individuals, even those in the lowest light intensity treatment, reacted clearly to the light at night introduced at the start of experimental period 2. Birds adjusted their activity patterns, with especially a strong response in the morning (Figure 6.1). During the following experimental period 3 with dark nights, activity patterns quickly reverted back to normal, with a very sharp on- and offset coinciding with the moments the daylight lamps were switched on and off, respectively, comparable to experimental period 1.

We analysed relative activity onset, relative activity offset, total activity and nocturnal activity in order to quantify the behavioural responses of our birds. The interaction between night light treatment and experimental period was highly significant for all activity variables, meaning that the effect of light intensity treatment depended on the experimental period (Table 6.1). For relative activity onset, offset and nocturnal activity, we detected a significant effect in period 2, the period where light at night was provided, but not in periods 1 and 3 (control periods; no light at night, all treatment groups equal). For total activity, there was a treatment effect only in experimental period 1 where for unknown reasons the birds that were going to receive the 0.15 lux treatment in period 2 had a lower total activity than the other experimental groups.

Effects of light intensity treatment on onset of activity were largest with highest light intensities. The birds that were exposed to 0.05 lux white light at night started their activity about half an hour before daylight was switched on, whereas birds that were exposed to 5 lux at night became active on average more than five hours before 'start of day' (Figure 6.2A). Although offset of activity was more variable, birds under the highest light intensities stayed active for about half an hour longer after daylights were switched off (Figure 6.2B). The OH test revealed that both onset and offset of activity were ordered by light intensity (last column of Table 6.1). The proportion of total activity expressed during the objective night increased in experimental period 2 with increasing light intensity, from almost no activity at 0.05 lux up to half of their total 24 hour activity when exposed to nocturnal illumination of 5 lux (Figure 6.2D). Again, the OH test showed that the order in nocturnal activity levels was as expected from the increasing light intensity treatments. Birds did not change the total amount of time they were active in each

24 hour cycle in response to artificial light at night (Table 6.1, Figure 6.2C); those with higher activity levels at night reduced their activity during the day.
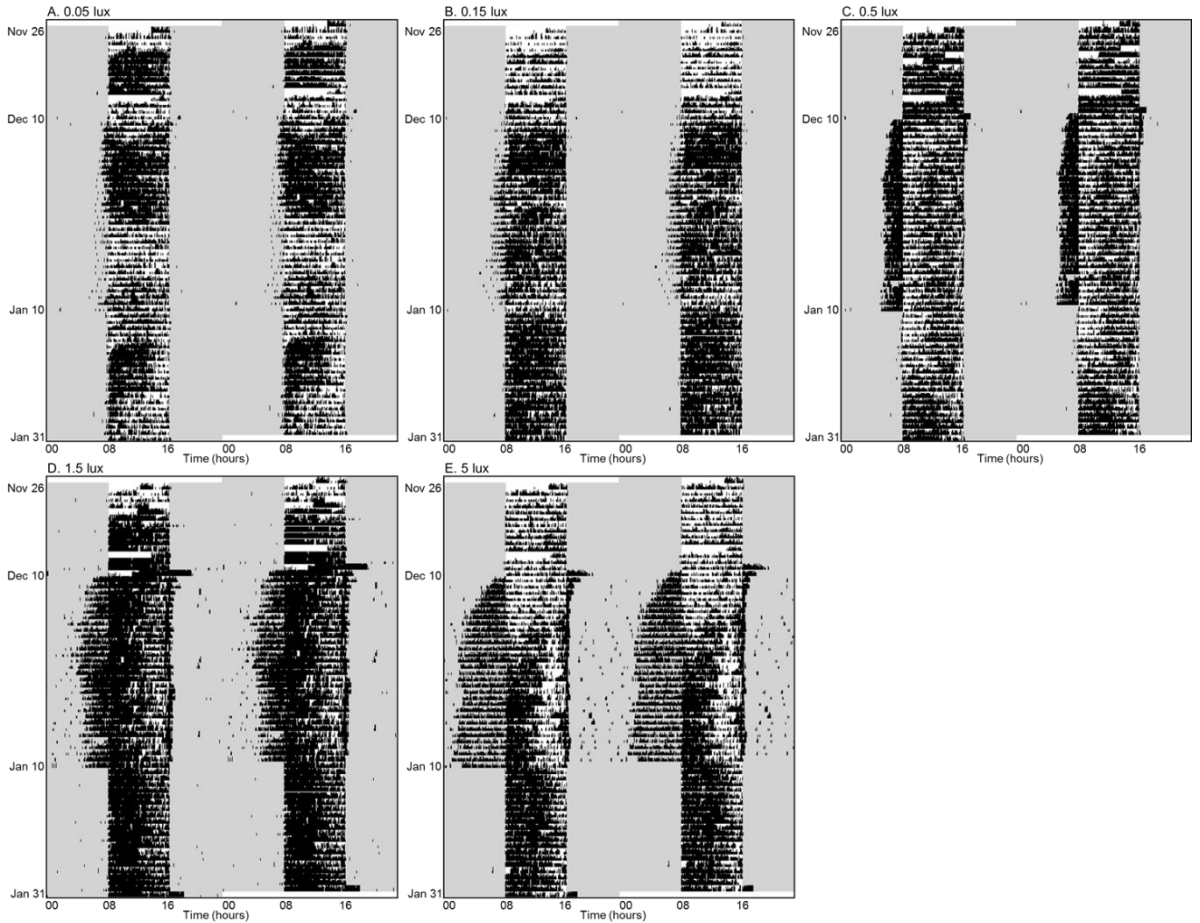

Figure 6.1 Double plotted actograms of five representative individuals, one from each treatment group (0.05 lux (A), 0.15 lux (B), 0.5 lux (C), 1.5 lux (D) and 5 lux (E)). Each actogram shows the activity of one individual bird and each row represents two consecutive days. Black bars represent activity in each two minute bin, where height of the bar is proportional to the amount of activity. Grey areas indicate when daylight lamps were switched off (between 16:45h and 8:30h). Dates at the y-axis give start and end of each of the periods; experimental period 1: 26 November 2013 – 10 December 2013 (night light lamps off); experimental period 2: 10 December 2013 – 10 January 2014 (night light lamps on); experimental period 3; 10 January 2014 – 31 January 2014 (night light lamps off).

Table 6.1 Results of the linear-mixed-effects models (LMMs) on four activity and three melatonin response variables, for the effect of night light intensity in the three experimental periods. The model output for the light intensity treatment with experimental period interaction term and, if the interaction term was not significant, for the treatment and experimental period, main effects are given in the overall analysis column (backward selection: least significant term was taken out of the model first, statistics are given for the step of the backward selection before the term was taken out). The sample size (n), the numerator and denominator degrees of freedom (ndf, ddf), the *F*-test statistic (*F*) and the significance level (*p*) are given for each term, significant *p*-values (<0.05) are indicated in italics. For the post-hoc analysis (performed when the interaction term was significant), the chi-square test statistic (χ^2) and the significance level are given, *p*-values were Bonferroni corrected for multiple testing. In the last column we report the *rsP*c statistic and significance level for the ordered heterogeneity test.

		Overall analysis				Post-hoc analysis				
							Treatment		OH test	
		n	ndf, ddf	*F*	*p*		χ^2	*p*	*rsP*c	*p*
Relative activity onset	Treatment : Exp. period	1404	8, 1360	336.72	*<0.001*	Exp. period 1	0.38	1		
						Exp. period 2	548.76	*<0.001*	1.00	*<0.0001*
						Exp. period 3	0.08	1		
Relative activity offset	Treatment : Exp. period	1438	8, 1396	5.11	*<0.001*	Exp. period 1	0.50	1		
						Exp. period 2	19.81	*<0.01*	0.59	*0.04*
						Exp. period 3	2.82	1		
Total activity	Treatment : Exp. period	1362	8, 1318	15.45	*<0.001*	Exp. period 1	21.68	*<0.001*		
						Exp. period 2	10.74	0.09	0.51	0.06
						Exp. period 3	6.46	0.50		
Nocturnal activity[a]	Treatment : Exp. period	1362	8, 1318	280.05	*<0.001*	Exp. period 1	2.28	1		
						Exp. period 2	242.46	*<0.001*	0.90	*<0.001*
						Exp. period 3	2.12	1		
Midday melatonin level	Treatment : Exp. period	100	8, 57	0.55	0.82					
	Treatment	100	4, 23	0.94	0.46					
	Experimental period	100	2, 65	0.30	0.74					
Midnight melatonin level	Treatment : Exp. period	101	8, 57	3.02	*<0.01*	Exp. period 1	2.19	1		
						Exp. period 2	20.68	*<0.01*	0.89	*<0.01*
						Exp. period 3	0.68	1		
Morning melatonin level	Treatment : Exp. period	100	8, 57	0.57	0.80					
	Treatment	100	4, 24	0.27	0.90					
	Experimental period	100	2, 65	3.41	*0.04*[b]					

[a] Proportion of total activity that took place during the night, nocturnal activity, was arcsine transformed before analysis.

[b] Experimental period 1: 147 ± 22.6 ng/ml; experimental period 2: 104 ± 20.5 ng/ml; experimental period 3: 180 ± 20.3 ng/ml (mean ± s.e.).
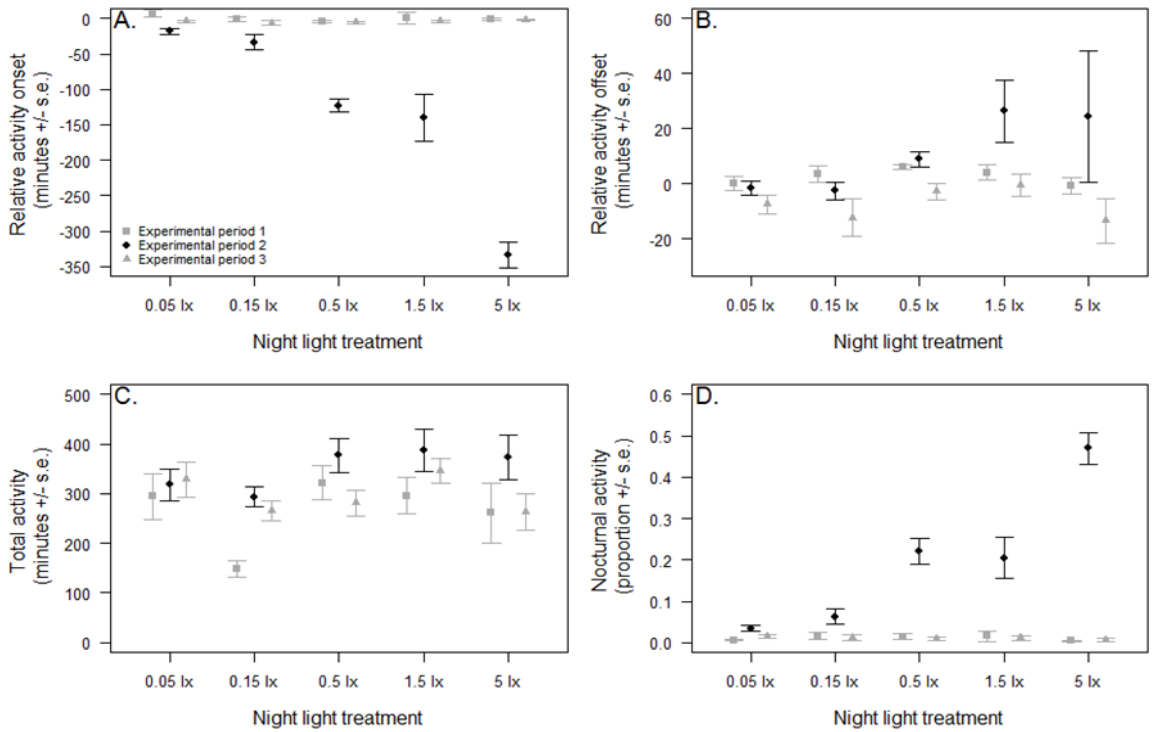
Figure 6.2 Behavioural response of great tits to five night light intensity treatments. Grey squares indicate experimental period 1 (no light at night), black circles indicate experimental period 2 (with night light treatment) and grey triangles indicate experimental period 3 (no light at night). Data has been averaged over individuals, for graphical purposes only. A. Onset of activity in minutes relative to 'start of day' (minutes after daylight was switched on). B. Offset of activity in minutes relative to 'end of day' (minutes after daylight was switched off). C. Total active minutes in 24 hour cycle. D. Nocturnal activity; part of the total activity that took place during the night (from daylight off till daylight on; note that this variable was arcsine transformed in the data analysis and presented here without the transformation).
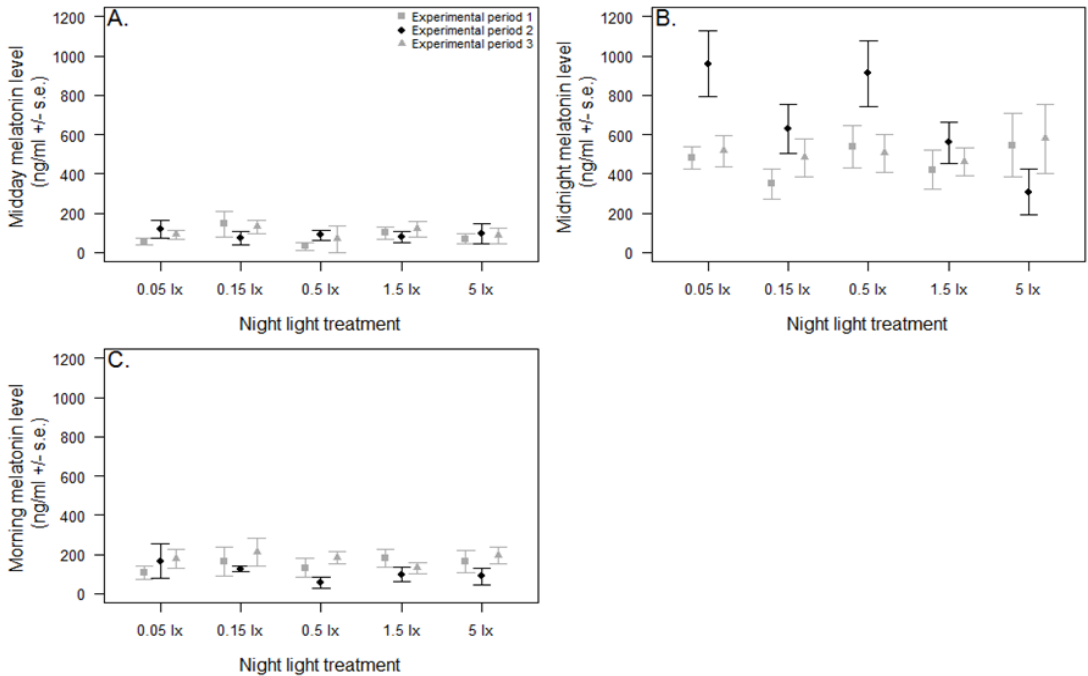
Figure 6.3 Response on plasma melatonin concentrations of great tits for five night light intensity treatments. Grey squares indicate experimental period 1 (no light at night), black circles indicate experimental period 2 (with night light treatment) and grey triangles indicate experimental period 3 (no light at night). A. Plasma melatonin level (ng/ml) sampled at midday (between 12:30 and 13:30). B. Plasma melatonin level (ng/ml) sampled at midnight (between 00:00 and 01:00). C. Plasma melatonin level (ng/ml) sampled in the early morning (between 06:30 and 07:30).

6.3.2 Melatonin levels

Plasma melatonin concentrations were measured at midday, midnight and in the early morning at the end of each experimental period. Results for midnight melatonin levels followed our expectations; the effect of treatment differed per period, with decreasing levels with increasing night light intensity in period 2, where the OH test showed that the order in midnight melatonin data followed the order of light intensity treatment groups (Table 6.1, Figure 6.3B). In the dark nights of periods 1 and 3, however, we found no effect as all individuals had elevated melatonin concentrations. In midday melatonin levels, we found neither an effect of treatment or experimental period, nor of the interaction between these two (Table 6.1, Figure 6.3A). Early morning melatonin levels were back to baseline (daytime) levels, comparable to those measured at midday, in all experimental periods. Therefore it was not surprising that we did not find a light intensity by

experimental period interaction effect and no effect of the light at night treatment in the second period on morning melatonin levels, although melatonin concentrations in the morning were in general slightly lower during experimental period 2 (Table 6.1, Figure 6.3C).

6.4 Discussion

In this study, we show a strong dose-response relationship for the effect of night light intensity on activity patterns and physiology in the great tit. We found a strong response in the daily onset of activity. Birds advanced their activity more when exposed to higher light intensities at night, the part of their active period that took place during the objective night increased with higher intensities and they stayed active longer at the end of the day. Furthermore, naturally elevated night-time levels of the hormone melatonin decreased in a dose-response manner with more light at night. When treatment reversed back to control dark nights, activities and physiology returned to normal and there was no evidence of carry-over effects.

Our experimental assessment of a dose-response relationship in the laboratory is in line with field studies on blackbirds (Dominoni et al. 2014) and American robins (*Turdus migratorius*; Miller 2006), which show a correlation between artificial light level at night and onset of activity. We have now shown this effect experimentally with a wider range of light levels and found that relative activity onset was affected very strongly by light at night, whereas the effects on activity offset are more limited. The clear coincidence of activity onset with the switch from darkness to daylight, but the lack of coincidence of activity offset with the change from light to dark in the two 'dark night' periods 1 and 3, indicates that light is a stronger cue for timing onset than for timing offset of daily activity. As a result, activity offset was much more variable between individuals, confirming a general pattern observed in songbirds.

The total amount of activity during a 24 hour cycle did not change in response to exposure to light at night. In contrast, birds spread their activity over their full subjective day (the duration of the active phase increased, and the duration of the rest phase decreased). This is clearly visible by the increase in proportion of nocturnal activity with increasing light levels; birds did not only start activity earlier in the night phase, they also showed an increase in the amount of activity during the objective night and thus a decrease during the objective day.

The shift in onset of activity of up to five hours under the brightest conditions, is very large compared to results from earlier studies, for example city blackbirds advanced activity at maximum by one hour (Dominoni et al. 2014) and songbirds close to streetlights by two hours (Kempenaers et al. 2010). This could be due to the light intensity, which is not known exactly for these two earlier studies, but presumably lower than 5 lux. Another reason could be that our experiment was performed in winter with birds kept in short days (photoperiod 8:15L–15:45D). This means that they have only a short window of time to forage and obtain enough energy for the long nights. The extra light at night could provide the opportunity to start foraging earlier, before daylight, to spread their 'workload' over a longer time span.

Besides the effects on activity onset and nocturnal activity, birds under higher light intensities are also more active throughout the night, relative to their total activity. Thus, the activity prior to daylight on was a substantial amount of their overall daily activity. This is probably also what causes the large variability in activity offsets. In a natural situation, artificial light at night may increase visibility of birds for predators, thus increasing predation risk (Miles et al. 2013). A response to these predators may be by increasing alertness and thus restlessness. Although earlier studies have shown that birds use artificial light at night to extend their foraging activity into the night (Stracey et al. 2014; Russ et al. 2015), in our case we do not know the nature of the nocturnal activity, this may be caused by feeding behaviour or restlessness due to the presence of dim light.

Another interesting finding is the transition of the daily activity pattern from the first period, without light at night, into the second period, with light at night. The level of activity at night shows a gradual increase, and the onset gradually advances for almost every bird. It takes several days before these two parameters stabilise (see actograms of individual birds in Figure 6.1).This is particularly clear for the birds in the higher light treatments. The pineal gland, which produces melatonin, stores information about the photoperiod and might compare stored and received photoperiod every day which could cause a gradual shift in daily activity phase (Gwinner and Brandstätter 2001). Contrastingly, the transition from the second to the third period, in which they went back to dark nights, was immediate for all birds. A gradual return may be masked here by direct suppression of activity by darkness (Redlin 2001).

In our study, we show that birds' daily rhythms, in activity as well as melatonin, are influenced by low levels of light at night. The effects on activity patterns could be direct, or could be via effects of light at night on melatonin; however, we cannot separate these pathways. The measurements of melatonin that we used in this

study were taken at three different time points. Therefore, it was not possible to create a full melatonin profile for a 24 hour cycle for our birds. We were unable to take more than three samples of each bird in each experimental period and chose to measure at midday, because we wanted a baseline daytime, level for comparison, and at midnight and in the early morning because at those time points previous studies had found clear effects of light at night (Singh et al. 2012; Dominoni et al. 2013b).

Unsurprisingly, daytime melatonin concentrations were not affected by light treatment and these were assumed to be the minimum levels in the 24 hour cycle. What we did not expect is that early morning concentrations were also not affected by light intensity, however, they were on average slightly lower during experimental period 2. Because of the advancement of onset of activity during experimental period 2, most birds were active already at the moment the early morning samples were taken. Therefore, their melatonin levels could have been back at daytime baseline levels whereas during nights without light, they would come back to baseline around the time of sampling (see also Appendix Figure 6.A3).

Midnight samples revealed a strong effect of night light intensity: levels of melatonin decreased with increasing light levels. We hypothesized that, in general, melatonin secretion at night would be suppressed by providing night-time illumination to the birds; however, our results do not fully confirm this. Instead, for the lowest light intensity treatment, levels were higher in experimental period 2, compared to periods 1 and 3. When evaluating the effect of the experimental period in each of the light intensity treatments in a post-hoc analysis, we found that midnight melatonin levels were increased in experimental period 2 with illuminated nights compared to the experimental periods 1 and 3 with dark nights in birds under 0.05 lux (χ^2=14,40; p<0.01) and in birds under 0.5 lux (χ^2=11,59; p=0.02), but not in the other treatment groups (no differences between experimental periods, all p>0.05). Schoech et al. (2013) found a similar result for night-time levels with light at night of 3.2 lux and had no clear explanation for this.

The daily expression of melatonin in birds consists of baseline levels at daytime and elevated levels at night (Kumar et al. 2000a). In our experiment, during periods 1 and 3 with dark nights, it might be that at midnight, we measured melatonin before it had reached peak levels. In contrast, when exposed to light at night, the birds had advanced onset of activity. We hypothesize that also the daily rhythm of melatonin secretion could have advanced, as is shown to be the case in (human) shift workers (Dumont et al. 2001). Therefore, under illuminated conditions, we may have sampled melatonin levels from different phases of the

nocturnal peak. The midnight measurements of the 0.05 lux birds could represent melatonin levels around the peak (higher levels compared to dark nights), whereas the midnight measurements of the 5 lux birds could represent levels measured after the peak, when secretion is on its decrease (lower levels compared to dark nights). We want to stress that this is a post-hoc explanation (described in more detail in Appendix Figure 6.A3), which needs further testing, for instance by sampling many times per night. However, the results of earlier mentioned studies (Singh et al. 2012; Schoech et al. 2013) could potentially also be explained by this hypothesis.

The dose-response relationships between the on- and offset of activity, nocturnal activity and midnight melatonin level, and intensity of artificial light at night do not reveal a light intensity threshold. All relationships are gradual, although not linear. The range in light intensity from 0.05 to 5 lux is comparable with light levels regularly found in natural or rural areas, e.g., where a road is illuminated (Spoelstra et al. 2015). Directly underneath a lamp post with the same spectrum white LED light that was used in this study, light intensities up to 10 lux can be measured. However, light levels exponentially decrease with distance from the lamp post and at a distance of 25 meters, intensity is only around 0.01 lux (see Figure S1 of de Jong et al. 2015). This decrease means that in a natural situation, it should be easy for birds to escape the potentially strong disruption of their biological rhythm: they are mobile species and could easily move away from the light, within a short distance of the light source. Although moving away some 25 meters from a light source might help avoiding effects on daily rhythms, there may still be effects on other aspects of a birds' behaviour.

Birds are very mobile species, but for less mobile or sessile species this may be completely different, as they cannot move away from a light source that easily. Also, we now looked at this effect from a resident species point of view, but it might very well be that roads or other linear illuminated objects in a rural area are much more disturbing for nocturnally active species, such as bats (Stone et al. 2009) or migrating birds (Ronconi et al. 2015). We have shown that daily rhythms of birds can be strongly affected by nocturnal illumination and that these effects are dose-dependent, but also that effects become weak when light levels decrease fast at short distances from light sources. This might suggest that impacts may be easily avoided in a natural situation. Higher levels of artificial light at night in urban areas are omnipresent (Dominoni et al. 2014) and thus make it less easy for birds to avoid circadian disruption.
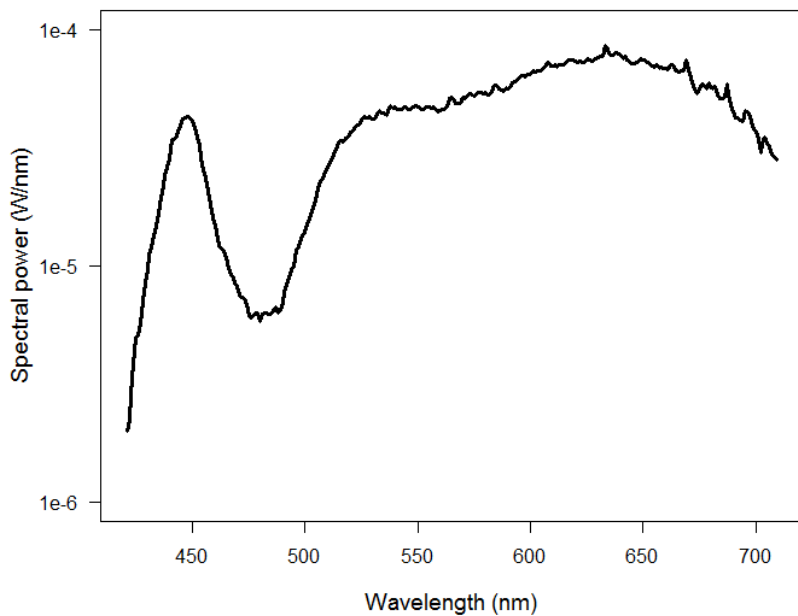
This study is the first experimental demonstration of dose-dependent effects of artificial light at night on avian daily activity patterns and melatonin levels. Our

results imply that nocturnal light effects are not limited to effects above a certain threshold, but are apparent even when light levels are slightly increased. This disruption in daily rhythms is especially relevant in urbanised areas, where light levels are increased over large areas, and can have potentially negative effects for an animal's fitness. Future studies should focus on the effects of different intensities of light at night in the wild. Our findings improve our understanding of the impacts of artificial light at a mechanistic level but also offer researchers and conservationists valuable information on intensity-dependent effects of artificial light at night.

Acknowledgements

Appendix

Figure 6.A1 Spectral composition of one of the 5 lux warm white LED lamps (Philips, Eindhoven, the Netherlands).
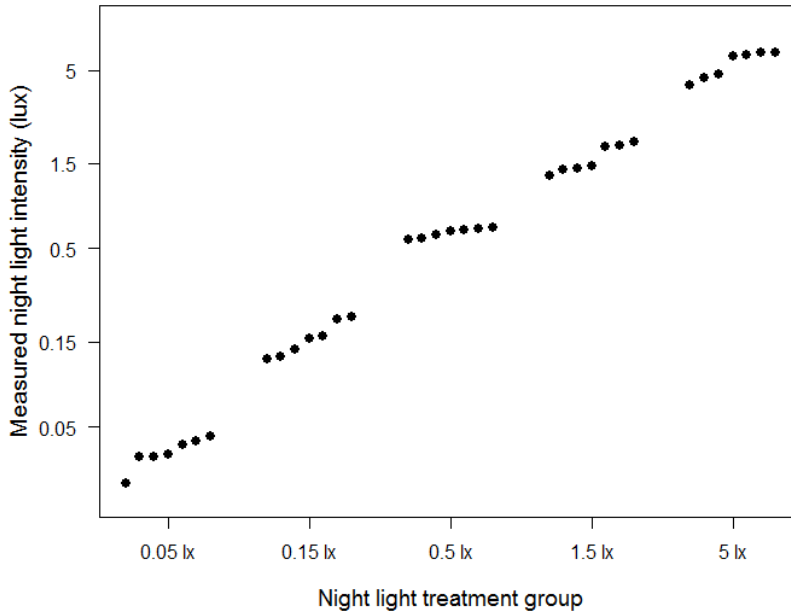
Figure 6.A2 Average of the night light intensity measured (in lux) at the two perches in each of the 35 cages, grouped by five night light treatment groups. Measurements were done with LMT B 360 S illuminance meter (LMT Lichtmesstechnik GmbH, Berlin, Germany).
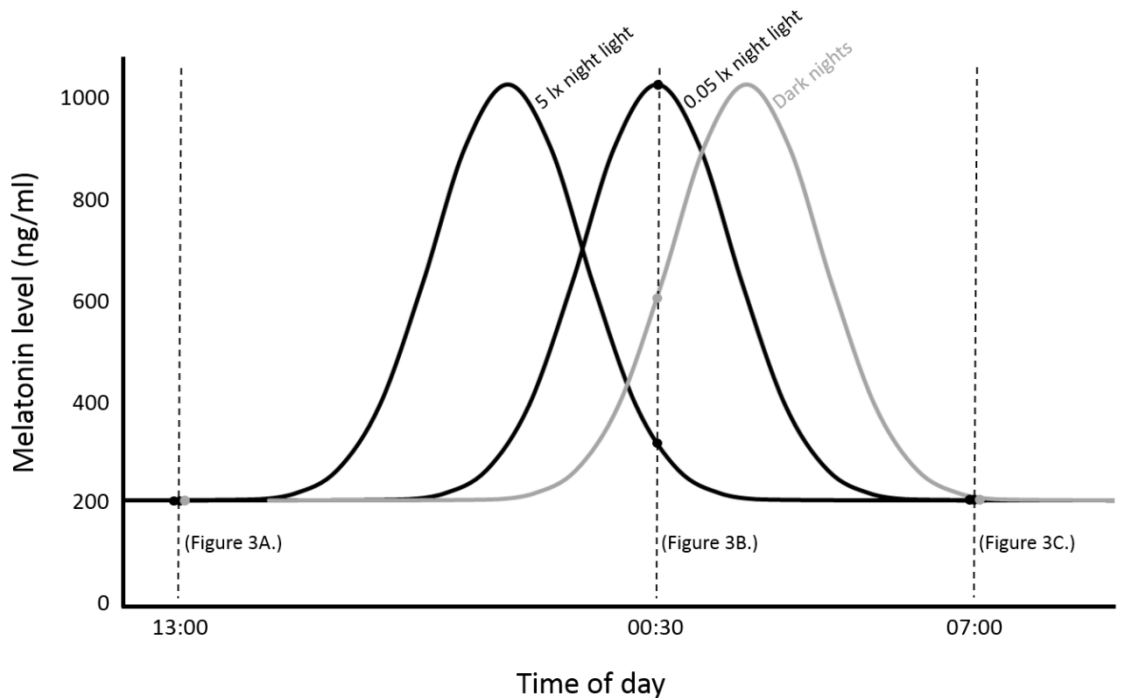
Figure 6.A3 Possible post-hoc explanation of daily profile of plasma melatonin level during experimental periods 1 and 3 (in grey) with dark nights and experimental period 2 (in black) with illuminated nights, according to light intensity treatment, here shown for 0.05 lux and 5 lux. The daily rhythm of melatonin secretion is hypothesized to shift as an effect of artificial light at night, following the advancement in onset of activity (see Figure 6.2A). The peak in melatonin level which normally, during dark nights, would be reached after midnight, might now be advanced and reached around midnight (0.05 lux) or even much earlier in the evening (5 lux). The vertical dashed lines correspond with the three moments that we sampled our birds; midday (12:30 – 13:30, see Figure 6.3A), midnight (00:00 – 01:00, Figure 6.3B) and early morning (06:30 – 07:30, Figure 6.3C).
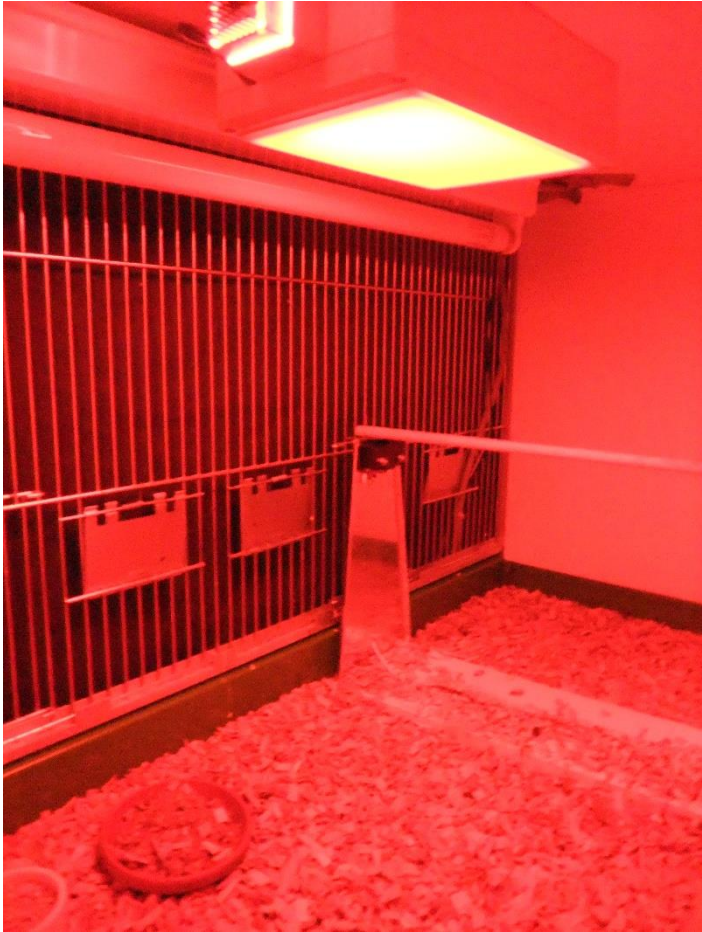
Chapter 7

Early birds by light at night: effects of light colour and intensity on daily activity patterns in blue tits

Maaike de Jong
Samuel P. Caro
Phillip Gienapp
Kamiel Spoelstra
Marcel E. Visser

Summary

1. The current change to LED outdoor lighting has rendered the use of coloured lighting feasible, and if the biological impacts of light colour differ in disturbance between colours, this opens the possibility to use light colours which affect the natural world to a lesser extent. One well known effect of artificial light at night is the disturbance of daily rhythms in birds. However, little is known about the effects of light of different colours on these rhythms and there are no studies so far on intensity-dependent effects of coloured light.

2. Here, we focus on effects of light colour and light intensity on daily activity patterns in a common songbird, the blue tit (*Cyanistes caeruleus*). The spectral composition we use is suitable for outdoor lighting and the light intensities are in the range of values to be found in a field situation. In a first experiment, we studied the activity patterns under green, red and white light at night, and compared these to a dark control. In a second, follow-up experiment, blue tits were exposed to different intensities of green and white light at night.

3. The birds advanced their onset of activity in the morning under all light colours, but most in red and white light. Offset of activity was slightly delayed in all light colours. The total activity of birds over a 24 h period did not change due to light, but part of their daily activity was moved into the night, proportional to the advancement of onset. The effect of light intensity on activity onset was smaller in green than in white light in the lower range of intensities, but became equal in the highest intensities. Results for offset, total activity and nocturnal activity were consistent with the first experiment.

4. *Synthesis and Applications.* These two experiments show that there are differences in effects of light at night between light colours and that green light, at low intensities, has a less disturbing effect on daily rhythms in blue tits. Further studies will determine whether the disturbance of daily activity patterns by light at night has any effects on individual reproductive success or survival, but our results show that the strength of the disturbance can, to a moderate degree, be mitigated by changes in characteristics of outdoor lighting.

7.1 Introduction

Light pollution is defined as the alteration of natural light levels in the outdoor environment owing to artificial light sources (Cinzano et al. 2000). Light pollution is not only a problem for humans, as the night sky brightness damages our perception of the starry sky (McNally 1994) and can have severe impacts on human health (Cho et al. 2015), but artificial light at night can also have strong impacts on wildlife (Rich and Longcore 2006; Hölker et al. 2010). In the Western world, there are few places left where nights are truly dark (Cinzano et al. 2001); the natural light-dark cycle under which animals evolved is disturbed by anthropogenic light at night.

Birds, for example, align their activities to the appropriate time of the day and year, through stimulation of photoreceptors by daylight to synchronize their internal circadian and circannual clocks (Dawson et al. 2001). Recent studies on bird populations in the wild have shown that artificial light at night can affect many aspects of a bird's life: blackbirds (*Turdus merula*) extend foraging activity into illuminated nights (Russ et al. 2015), timing of dawn singing of common songbirds is altered (Kempenaers et al. 2010; Da Silva et al. 2014) and timing of reproduction in blue (*Cyanistes caeruleus*) and great tits (*Parus major*) is advanced by light at night (Kempenaers et al. 2010; de Jong et al. 2015). In captivity, the effects of nocturnal illumination on avian daily rhythms have been studied in more detail. Locomotor activity was higher in blackbirds under light at night and increased sharply before dawn, when melatonin levels were decreased compared to birds under dark nights (Dominoni et al. 2013b). In great tits, activity onset was increasingly advanced, and activity offset delayed (de Jong et al. 2016).

The research interest in the biological impacts of artificial light at night has grown enormously over the last years (Gaston et al. 2015), and options to reduce the effects of night-time light pollution on ecosystems are being investigated. Simply illuminating the environment less would obviously reduce these effects greatly, and also save energy costs, but might not always be feasible. Adaptation of the light spectrum is another option (Gaston et al. 2012). The use of modern light-emitting diode (LED) lamps in outdoor lighting has economic advantages, but, more importantly, their colour composition can be custom designed, potentially allowing for the mitigation of impact of light on flora and fauna via adaptation of the spectrum (Spoelstra et al. 2015). Ultimately, it should be possible to advise the use of lamps with certain characteristics (such as spectral composition and light intensity) for a specific setting, in such a way that the emitted light has the sufficient quality to support human activities and, at the same time, has minimal

effects on biological processes of the organisms or communities nearby (Musters et al. 2009).

To be able to eventually apply scientific results for advice on outdoor lighting, light levels should be standardized in lux, a measure of illumination based on human vision (lamps of different colours with the same intensity in lux are perceived by humans as equally intense). Lamps with different spectra will however differ in intensity for birds as the spectral sensitivity of birds differs from that of humans. Birds are sensitive to a range of wavelengths to which humans are blind (Bennett and Théry 2007). Many birds are UV sensitive and most birds perceive colours through four single cone types (Bennett and Cuthill 1994; Osorio and Vorobyev 2008). Also, they have extra-ocular light perception by photoreceptors in the pineal gland and brain (Cassone 2014). Hart et al. (2000) measured the spectral absorption characteristics of the photoreceptors in the eyes of the blue tit and the blackbird, which are very similar to those described in other passerines. There are small differences in the properties of their cones which may reflect differences in the visual ecology of these two species (Hart et al. 2000). Using lamps with different spectra can thus already differ in the effect they have on birds simply because some colours are perceived as less intense.

In addition to the perceived intensity of lights with different spectra by birds, some light colours may affect birds to a lesser extent than others, even when perceived at the same intensity, as the visual system may not predict to what extent different colours of light affect different kinds of behaviour. In fact, little is known about these effects. In general, long wavelengths are known to penetrate the skull more easily than short wavelengths, and have been found to be more effective at inducing a photoperiodic response (Hartwig and van Veen 1979), stimulating gonadal development and promoting body fattening than shorter wavelengths (Malik et al. 2002). Yadav et al. (2015) showed that blackheaded buntings (*Emberiza melanocephala*) and Indian weaverbirds (*Ploceus philippinus*) interpreted short (blue) and long (red) light wavelengths applied at equal energy levels as the day and night, respectively. Some studies showed that nocturnally migrating birds are disoriented by, and attracted to, white and red, but less to green and blue light (Wiltschko and Wiltschko 1995; Poot et al. 2008; but see also Evans 2010). In an experimental study on wild great tits, it was found that birds nesting in white illuminated areas or close to red lamps had elevated stress hormone (corticosterone) levels (Ouyang et al. 2015). All in all, results so far are not very consistent and it is not yet clear how light with different spectral composition affects the behaviour and fitness of birds.

In a previous study, we have shown that nocturnal light effects on daily rhythms in birds increase progressively with light intensity and are not only apparent once a threshold is reached (de Jong et al. 2016). This means that it is important to study intensity-dependent effects of artificial light. The effects of light at night with different colours on daily activity patterns are so far unknown, let alone the interaction between light intensity and colour. Therefore, in the current study we focussed on both the effects of light colour and light intensity on daily rhythms in a common songbird. We made use of light colours that are suitable for application in outdoor lighting (green, red and white light, see Spoelstra et al. 2015). In a first experiment, we studied the activity patterns of blue tits under green, red and white light at night, compared to a dark control. Based on the results of this experiment, we performed a second experiment, in which blue tits were exposed to different intensities of green and white light at night.

7.2 Methods

7.2.1 Birds

In the two experiments described here, we studied 28 and 35 male blue tits, respectively. All birds originated from Corsica and hatched in spring 2011. They were then moved to the Netherlands where they were hand-reared and housed at the Netherlands Institute of Ecology (NIOO-KNAW), following the procedure described in detail in Reparaz et al. (2014). During the experiments, birds were housed in individual cages (90 cm × 50 cm × 40 cm). Each cage had two separate light sources for day- and night-time illumination, and external light was completely excluded using covers that were placed on the cage fronts. Cages were therefore tight to light coming from outside or from other cages. Cages were ventilated and temperature was maintained around 15 °C, and did not vary structurally between day- and night-time. Birds had access to food and water *ad libitum*. During both experiments, birds were kept on constant 14 h light – 10 h 'dark'. To reduce possible disturbances by outside noise and neighbouring birds, white noise was played continuously on a low level. All experimental procedures were carried out under licences NIOO 12.16 and NIOO 13.11 of the Animal Experimentation Committee of the Royal Netherlands Academy of Arts and Sciences.

7.2.2 Experimental set-up

In both experiments, the individuals were assigned randomly to a treatment group and to one of seven blocks of cages, each block containing all night light treatments. These blocks were divided over two rooms. Treatments were allocated to cages using a Latin Squares design. Birds from the same families (i.e. brothers and sisters) were distributed evenly across treatment groups. During

daytime, 6:00 h – 20:00 h, birds were exposed to full spectrum daylight high frequency fluorescent lights emitting ± 1000 lx at perch level (Activa 172, Philips, Eindhoven, The Netherlands). During night light treatment periods, night light LED lamps were switched on at 19:45 h and off at 06:15 h, with 15 minute overlap with the daylight lamps.

In the first experiment (experiment 1), we tested the effect of different light colours on blue tit activity. This experiment was performed in 2012, and consisted of two periods. During the first period ("control", 14 November – 21 November), birds were not exposed to any light at night. In the second period ("experimental", 21 November – 10 December), birds were exposed to either green LED light, red LED light or white LED light (Philips, Eindhoven, The Netherlands) of approximately 5 lx at perch level, or no light (dark control). There were no differences between the light intensities, in lux, of the green, red and white lamps ($F_{2,18}$=2.07, p=0.16). For spectral composition of the three colours, see Appendix Figure 7.A1. In short, all lights emit full spectrum light; green lamps have an increased blue and reduced red light emission, and red lamps have an increased red and reduced blue emission. All light colours have negligible UV emission (Spoelstra et al. 2015).

In the second experiment (experiment 2), we tested the effect of the interaction of light colour and light intensity on activity of blue tits. This experiment was done in 2014, as a follow-up of experiment 1, and consisted of five periods alternating between control (dark nights) and experimental treatments. Based on the results of the first experiment, we chose to compare green and white light. During the first period ("control", 14 April – 28 April), all birds were kept under dark nights. In the second period ("experimental", 28 April – 22 May), the birds were divided over five treatment groups with different light intensities: dark, 0.15 lx, 0.5 lx, 1.5 lx and 5 lx of green or white light. During the third period (control, 22 May – 6 June), all birds had dark nights. In the fourth period (experimental, 6 June – 30 June), they were exposed to night light of the other colour (so either first green, then white, or first white, then green), or to no light at night. And finally in the fifth period (control, 30 June – 8 July), all birds were kept under dark nights again. Spectral composition of the lamps in experiment 2 was equal to the green and white lamps of experiment 1.

7.2.3 Activity measurements
Daily activity patterns of each individual bird were measured continuously throughout both experiments. We used the same method as described in detail in de Jong et al. (2016). Briefly, each cage was equipped with one normal wooden perch and one wooden perch fitted with a microswitch that recorded perch-hopping activity. An on / off signal was registered every 0.1 s and software

developed by T&M Automation (Leidschendam, The Netherlands) created files in which each 30 second interval was logged. We obtained one level of activity per 2 min. In the analysis, we excluded the first five days of each experimental period, because birds had to acclimatize to their new environment or to the change of treatment. ChronoShop 1.1 (written by KS) was used to calculate four activity descriptors for each individual, in each 24 hour cycle. The onset of activity is defined as the first moment the activity exceeds the average activity of the day. In order to avoid 'premature' onsets, a running mean of 20 minute length was fitted in the raw activity data. Activity onset is reported in minutes relative to the moment the daylight was switched on, 'start of day'. The offset of activity, the last moment the activity was above the average activity value of the day, was calculated in the same way, and reported as minutes relative to the moment daylight was switched off, 'end of day'. Total activity is defined as the total number of minutes in which the bird has been active in a 24 hour cycle. Finally, nocturnal activity is calculated as the proportion of total activity that took place during the objective night (daylight off to daylight on). These four measures of daily activity were used as response variables in separate analyses.

7.2.4 Statistical methods

All statistical analyses were done using R v. 3.1.2 (R Development Core Team 2014) with a significance level of α=0.05 and all analyses were based on averages of the response variable per individual, per experimental period. For experiment 1, we used linear-mixed-effects models (LMMs) with treatment as fixed effect and block nested within room as random intercepts, to account for possible effects of the location of the cage, for all four response variables. First we tested for an effect of treatment group (a four level factor) in the first control period, with dark nights for all individuals, then we tested for an effect of treatment in the second period, with light treatment at night. For experiment 2, we used LMMs with individual nested within block, which is nested within room as random intercepts, to account for multiple measures of the individuals, for all four response variables. First, we tested for an effect of period for all individuals over the three dark night periods (period 1, 3 and 5), to check for possible seasonal effects, and for the individuals that were kept under dark nights during one of the experimental periods we compared those with the previous and next period (either period 1, 2 and 3 or period 3, 4 and 5), to check for carry-over effects of treatments or effects of neighbouring birds during experimental periods. Then, we used light colour (a two level factor), light intensity (a continuous variable) and their interaction as fixed effects to test for the effect of light treatment during the experimental periods 2 and 4. We used actual, measured light intensity as explanatory variable in our analyses instead of treatment as factor, because light intensities of the green and white LED lights differed (see for

measurements of all lamps Appendix Figure 7.A2). If the interaction between light colour and light intensity was significant, we subsetted the data in green and white light and tested for the effect of intensity. Statistical significance was tested with F-tests using Kenward and Roger adjustment (Halekoh and Højsgaard 2014). Significant covariates were kept in the models.

7.3 Results

Blue tits clearly reacted to the light at night in both experiment 1 and 2 (actograms of representative individuals from both experiments are given in Appendix Figure 7.A3). Birds adjusted their activity patterns, with a strong response in the onset of morning activity. In contrast, during the periods with dark nights, activity patterns were normal, with a sharp on- and offset coinciding with the moments the daylight lamps were switched on and off, and in control periods after experimental periods, patterns went quickly back to normal. We analysed relative activity onset, relative activity offset, total activity and nocturnal activity in order to quantify the behavioural response of the birds to artificial light at night.

7.3.1 Experiment 1
In the dark night period (period 1) of experiment 1, groups of birds did not differ in their onset ($F_{3,18}$=0.70, p=0.57), offset ($F_{3,18}$=0.79, p=0.51), total ($F_{3,18}$=0.12, p=0.95) and nocturnal activities ($F_{3,18}$=0.95, p=0.44). In experimental period 2, light colour treatment had a significant effect on the relative onset of activity: birds illuminated with red or white light at night advanced their onset by more than two hours, and birds in green light advanced their onset by more than one hour, compared to birds from the dark control group (Table 7.1, Figure 7.1A). Light colour treatments also significantly affected the relative offset of activity: birds in illuminated nights delayed their offset by approximately one hour compared to the dark control, independent of the light colour (Table 7.1, Figure 7.1B). Birds illuminated at night increased the proportion of total activity expressed during the objective night by 20%, compared to controls, when they were exposed to green light, and by up to 30% when they were exposed to red or white light (Table 7.1, Figure 7.1D). Birds with higher activity levels at night did however reduce their activity during the day, resulting in a similar total activity across treatments over each 24 hour cycle (Table 7.1, Figure 7.1C.).

7.3.2 Experiment 2
In experiment 2, birds exposed to green or white light at night changed their relative activity onset in a different manner with increasing light intensity. For both colours, activity was advanced more with increasing intensity. The effect was smaller in the green light than in the white light at low intensities, but increased

faster in green than in white as intensity increased (Table 7.2, Figure 7.2A). Relative activity offset was affected by light intensity in the same way for the two light colours; birds delayed their offset further into the night with increasing light intensity, up to half an hour in both green and white light (Table 7.2, Figure 7.2B). As in experiment 1, the increase in the daily activity that was moved into the night with increasing light intensity, was proportional to the advancement of onset in both light colours (Table 7.2, Figure 7.2D). Birds did not change the total amount of time they were active in each 24 hour cycle in response to light intensity or colour (Table 7.2, Figure 7.2C) and variation in total activity was very large. By comparing the individual response variables over the dark night periods of experiment 2, we found no major seasonal effects, carry-over effects of treatments or effects of neighbouring birds during experimental periods, although there were some significant differences (see Appendix Figure 7.A4).

Table 7.1 Results of the linear-mixed-effects models (LMMs) on the four activity response variables, for the effect of night light colour in experimental period 2 of experiment 1. The model output for the treatment main effect is given. The sample size (n), the numerator and denominator degrees of freedom (ndf, ddf), the *F*-test statistic (*F*) and the significance level (*p*) are given for each term, significant *p*-values (<0.05) are indicated in italics. Tukey all-pair comparisons were done when treatment was significant.

Response	Explanatory	n	ndf, ddf	*F*	*P*
Relative activity onset	Treatment[a]	28	3, 18	24.71	*<0.001*
Relative activity offset	Treatment[b]	28	3, 18	6.00	*0.005*
Nocturnal activity	Treatment[c]	28	3, 18	19.52	*<0.001*
Total activity	Treatment	28	3, 18	0.99	0.42

[a] G-D, R-D, W-D, R-G *p*<0.001; W-G *p*=0.03; W-R *p*>0.05
[b] G-D *p*=0.01; R-D *p*=0.02; W-D *p*<0.001; R-G, W-G, W-R *p*>0.05
[c] G-D, R-D, W-D *p*<0.001; R-G *p*=0.04; W-G *p*=0.01; W-R *p*>0.05
p values are adjusted for multiple testing equivalent to adjusting significance levels following the Benjamini & Hochberg procedure, using the *p.adjust* in *glht* function within the *multcomp* package in R (Waite and Campbell 2006).
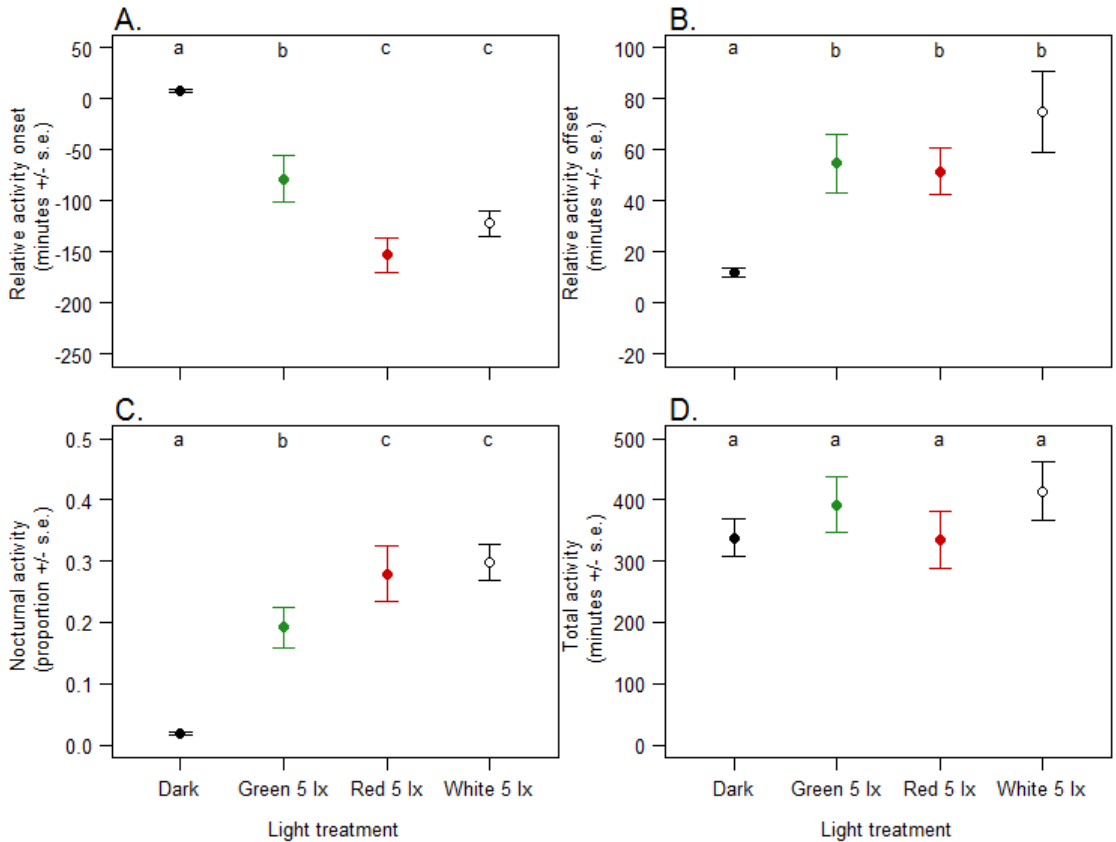
Figure 7.1 Behavioural response of blue tits to the four light colour treatments in experiment 1, experimental period 2. Data has been averaged over individuals. A. Onset of activity in minutes relative to 'start of day' (minutes after daylight was switched on). B. Offset of activity in minutes relative to 'end of day' (minutes after daylight was switched off). C. Nocturnal activity; part of the total activity that took place during the night (from daylight off till daylight on). D. Total active minutes in 24 hour cycle. Letters indicate the results of the Tukey post-hoc tests (see Table 7.1).

Table 7.2 Results of the linear-mixed-effects models (LMMs) on the four activity response variables, for the effect of night light colour and intensity in experimental periods 2 and 4 of experiment 2. The model output for the light colour with light intensity interaction term and, if the interaction term was not significant, for the light colour and light intensity main effects, are given (backward selection: least significant term was taken out of the model first, statistics are given for the step of the backward selection before the term was taken out). The sample size (n), the numerator and denominator degrees of freedom (ndf, ddf), the *F*-test statistic (*F*) and the significance level (*p*) are given for each term, significant *p*-values (<0.05) are indicated in italics. If the light colour with intensity interaction term was significant, the statistics for light intensity within green and white subsets and intercept and slope for this term are given.

Response	Explanatory	n	ndf, ddf	*F*	*p*	Intercept (s.e.)	Slope (s.e.)
Relative activity onset	Colour : Intensity	56	1, 44	7.62	*0.008*		
	Green : Intensity	28	1, 20	125.74	*<0.001*	11.06 (2.95)	-15.81 (1.41)
	White : Intensity	28	1, 20	38.10	*<0.001*	-3.38 (4.62)	-8.57 (1.39)
Relative activity offset	Colour : Intensity	56	1, 46	0.57	0.46		
	Colour		1, 28	0.06	0.80		
	Intensity		1, 48	36.15	*<0.001*	1.08 (2.66)	3.09 (0.49)
Nocturnal activity	Colour : Intensity	56	1, 39	11.58	*0.002*		
	Green : Intensity	28	1, 20	153.45	*<0.001*	-0.01 (0.007)	0.04 (0.003)
	White : Intensity	28	1, 20	50.86	*<0.001*	0.01 (0.010)	0.02 (0.003)
Total activity	Colour : Intensity	56	1, 36	0.26	0.61		
	Colour		1, 24	0.69	0.41		
	Intensity		1, 38	0.93	0.34		
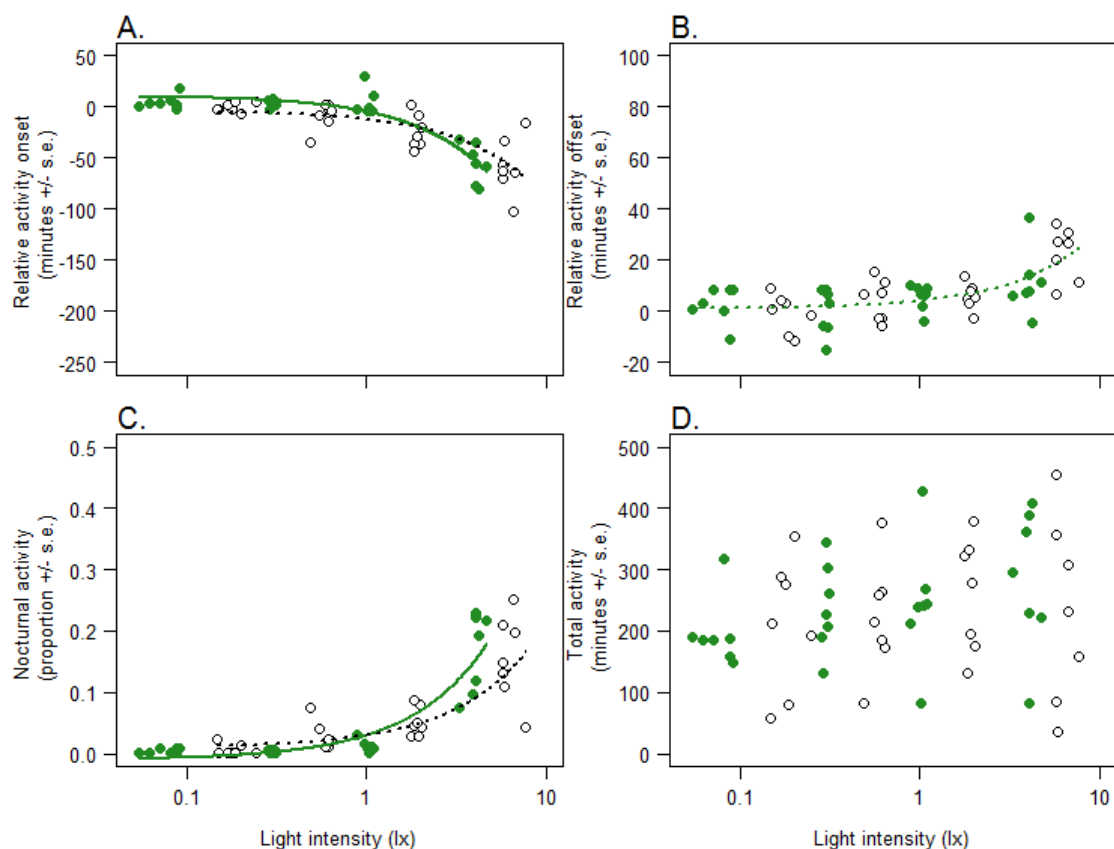
Figure 7.2 Behavioural response of blue tits to green and white light with different intensity, in experiment 2, during experimental periods 2 and 4. Data has been averaged over individuals. A. Onset of activity in minutes relative to 'start of day' (minutes after daylight was switched on). B. Offset of activity in minutes relative to 'end of day' (minutes after daylight was switched off). C. Nocturnal activity; part of the total activity that took place during the night (from daylight off till daylight on). D. Total active minutes in 24 hour cycle. Predictions of the LMMs with significant terms are plotted (see Table 7.2). Note that light intensity on the x-axis is plotted on a logarithmic scale.

7.4 Discussion

In experiment 1, birds' daily activity patterns were clearly disrupted. Most strikingly, their activity already started up to two hours before the day lights went on. This advancement of their onset of activity in the morning was present under all light colours, but most pronounced in red and white light. Their offset of activity was slightly delayed in all light treatments. The total amount of time that birds were active over a 24h period did not change due to light at night, but part of their

daily activity was moved into the night, proportional to the advancement of onset. In experiment 2, there was a clear dose-response relationship of change in activity pattern versus light intensity. Furthermore, we show that the effect of light intensity on activity onset was smaller in green than in white light in the lower range of intensities, but increased faster with increasing intensity in green light. Activity offset was delayed more with more green or white light and the results for total activity and nocturnal activity were consistent with those of experiment 1.

Although the general patterns are quite similar, there are some discrepancies in the results of the two experiments. Firstly, the strength of the response to light at night differed for what was basically the same treatment (green 5 lux and white 5 lux treatments). While in experiment 1 the birds advanced their onset by up to two hours, in experiment 2 this was only slightly more than one hour. Also the delay of offset was larger in the first experiment for the same treatments. Birds stayed active about an hour longer in the evening when daylight was switched off, while in the second experiment this was only around half an hour in the highest light intensities. Secondly, the difference in response of onset of activity to green and white light was not consistent between the two experiments. In experiment 1, green light clearly had less effect than white light, while in experiment 2 this was the case for the lower, but not for higher intensities of light (including the intensity used in experiment 1). The difference between green and white in the first experiment was the main reason that we chose to have these two colours in experiment 2 where we looked at light intensity (due to limitations in space and time, we could not have all three light colours). It is unclear where these discrepancies originate from; the set-up of both experiments, the experimental procedures and the spectral composition of the lamps were exactly the same. However, the experiments were done in different times of the year and birds were older in the second experiment.

A similar experiment was conducted in another passerine study species, the great tit (de Jong et al. 2016). Again, the experimental set-up, the procedures and the spectral composition and intensities of the lamps were the same. Like in blue tits, daily activity patterns of great tits showed a dose-dependent response to light at night. Under white light, both species progressively advanced their activity onset with increasing light intensity. However, great tits advanced faster and much more, up to five hours in the 5 lx treatment, whereas the maximum advancement of the blue tits under white light of 5 – 7 lx is around two hours. Although the spectral absorption characteristics of retinal photoreceptors measured in several bird species are broadly similar (Bowmaker et al. 1997), it may well be possible that great tits and blue tits do differ in their spectral sensitivity and action spectra, by differences in spectral transmission through oil droplets and ocular media, and

hence perceive the different light colours as different intensities. In fact, another experiment suggested that blue tits were less able to find their food than great tits, when kept under the same, very low light levels (Caro et al. unpublished data). For blue tits, the wavelengths of maximum sensitivity are known for the rods (503 nm), single cones (372 nm (ultraviolet-sensitive), 449 nm (short-wavelength-sensitive), 502 nm (medium-wavelength-sensitive) and 563 nm (long-wavelength-sensitive)) and double cones (563 nm (principal) and 565 nm (accessory)) (Hart et al. 2000), for great tits these are not yet measured. It does however not mean that the visual action spectrum of the blue tit directly explains changes in behaviour, i.e., it is not known how the relative abundance of light of specific wavelengths causes changes in for example daily activity patterns.

Apart from the bird species, photoperiod also differed between the two experiments: here we used 14L-10D, while the great tits were kept under shorter days, 8:15L-15:45D. The difference in advancement of activity could also be due to the difference in day length; under long nights and short days, birds have only a short time window to forage and obtain enough energy. The nocturnal illumination could provide an opportunity to start foraging already before daylight and to spread workload over a longer period (de Jong et al. 2016). Birds under long nights might therefore make use of the extra light at night much more compared to birds under shorter nights and longer days. The results in the advancement of onset of activity that we currently show in blue tits are in line with those earlier found in city blackbirds (1 h; Dominoni et al. 2014) and songbirds close to streetlights (2 h; Kempenaers et al. 2010).

Our finding that green light at low intensities disturbs the daily rhythm of blue tits less than white light at the same intensities (in lux), is in line with earlier studies that show that long wavelength light (red and white) penetrates the skull more easily (Hartwig and van Veen 1979) and has larger effects on gonadal development (Malik et al. 2002) and stress hormones (Ouyang et al. 2015). It remains unclear why we do not find this difference in higher light intensities and more studies are needed to provide a decisive answer about whether green light is truly less disturbing for birds. If this indeed turns out to be the case, a next step would be to see whether the effects on daily activity patterns have any fitness consequences, for example through disturbance of sleep (Steinmeyer et al. 2013; Raap et al. 2015). A field experiment in previously dark, natural areas, using lamps with the same spectral compositions, did not show clear, unidirectional effects of experimental nocturnal illumination on fitness so far, but underlines the need for further research on fitness consequences (de Jong et al. 2015).

The dose-response relationship of change in activity pattern versus light intensity which we show here, indicates that decreasing light intensity could be a measure to reduce the effects of artificial light at night. Gaston et al. (2012) indicated five main management options to reduce the effects of night-time light pollution, among which are changing the intensity of lighting as well as changing the spectral composition of lighting. The fact that the light spectra used in this study enable full colour vision for humans, because they cover a continuous and broad spectrum (Spoelstra et al. 2015), and that the range in light intensities used is comparable with light levels regularly found in natural or rural areas, for example near roads, makes our results suitable for application in outdoor lighting. We have shown that there are differences in effects on avian daily rhythms between light colours and that green light at low light intensities disturbs daily activity patterns less. This means that the strength of the disturbance of behaviour of wild birds can be mitigated by changes in the characteristics of outdoor lighting, which opens up possibilities for conservationists and landscape planners to use this in their daily practice.
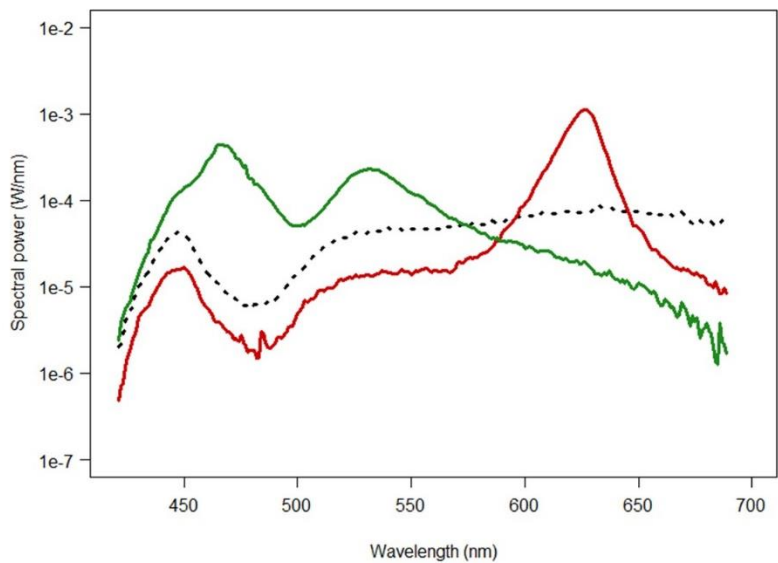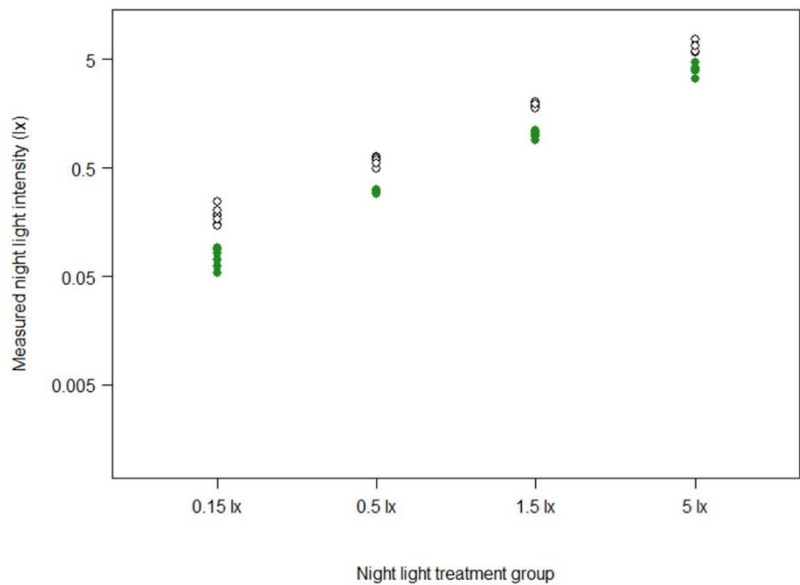
Acknowledgements

Data Accessibility

All data will be deposited in DataverseNL (to be made available after acceptance, doi will be added).

Appendix

Figure 7.A1 Spectral composition of the three light colours used in experiment 1. The green line corresponds to the green LED light, the red line to the red LED light, and the dashed line to the white LED light.

Figure 7.A2 Measured light intensities versus the 'original' light treatment groups of experiment 2. Green (closed) and white (open) dots correspond to the green and white LED lights, respectively.

A.
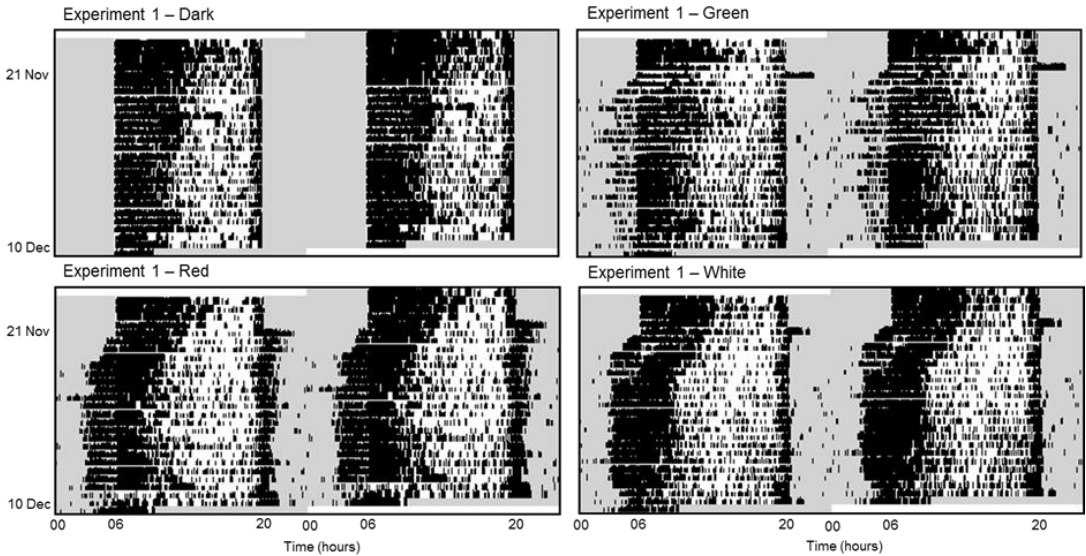
Figure 7.A3 Double plotted actograms of representative individuals from treatment groups of experiment 1 (A) and experiment 2 (B & C). Each actogram shows the activity of one individual bird and each row represents two consecutive days. Black bars represent activity in each 2 minute bin, where height of the bar is proportional to the amount of activity. Grey areas indicate when daylight lamps were switched off (between 20:00 h and 6:00 h). Dates at the y-axis give start and end of each experimental period. Treatment groups are indicated above the actograms. For experiment 2 this is the treatment group for experimental period 2 and experimental period 4, respectively (B & C).
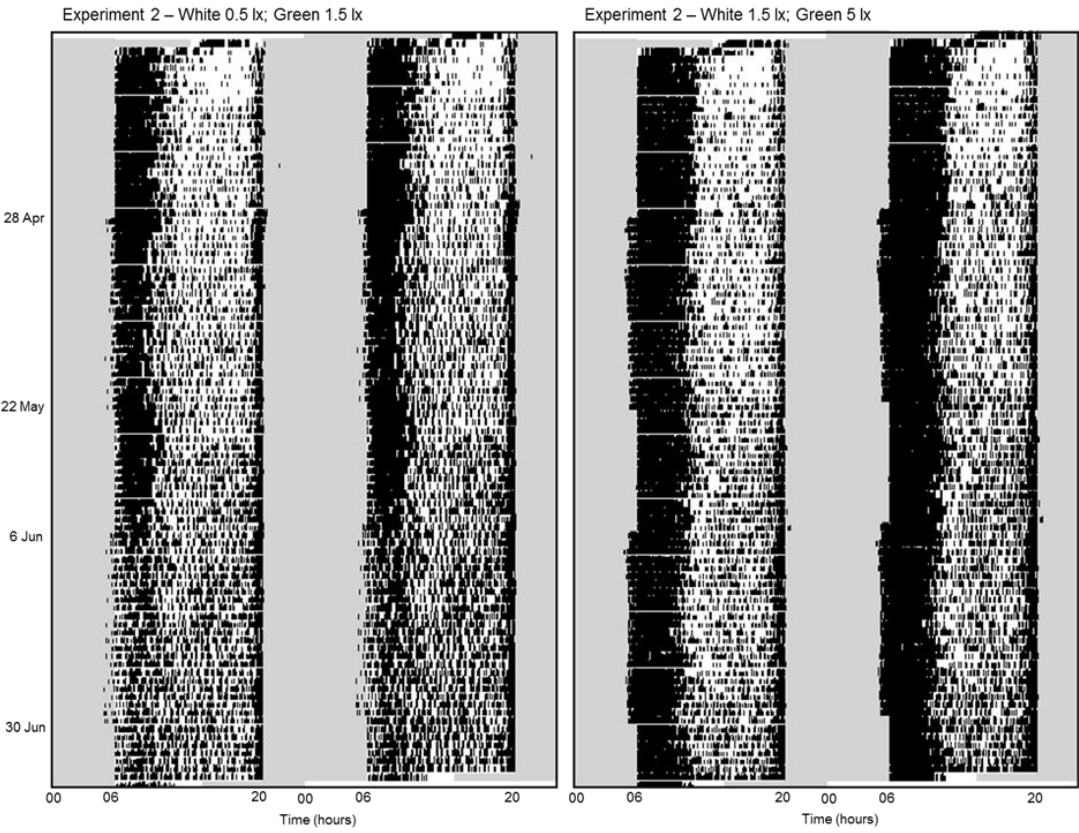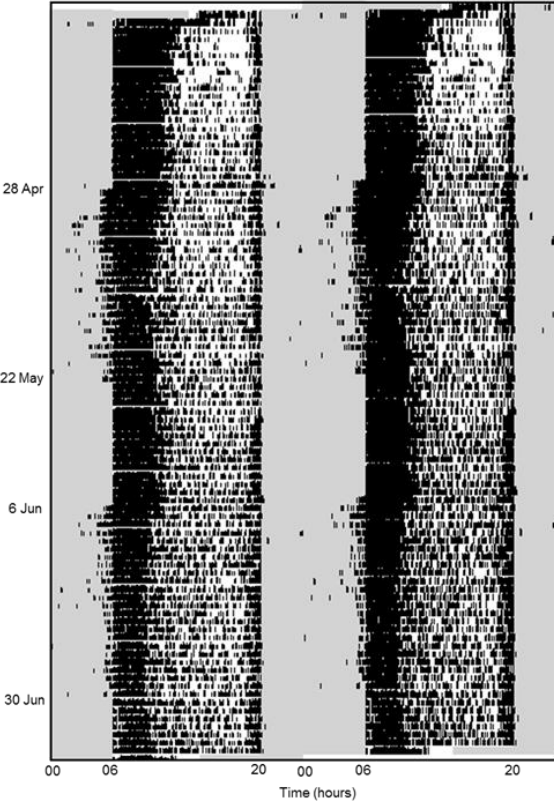
B.

Figure 7.A3 (*Continued.*)

C.
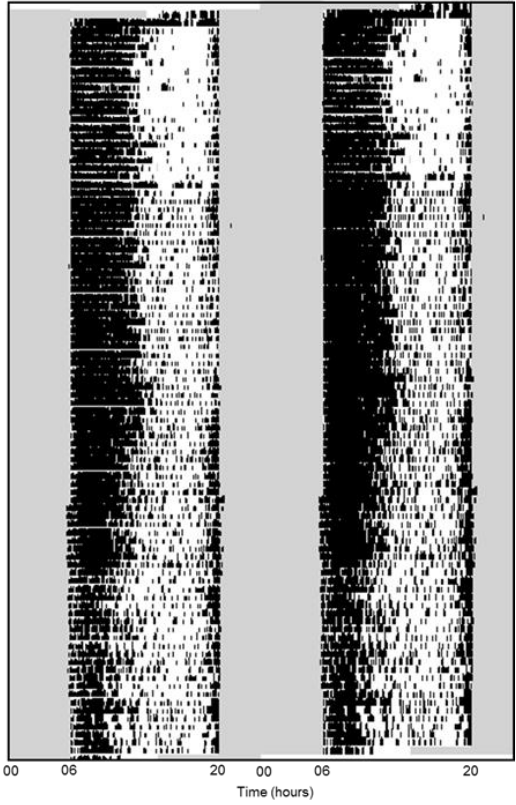
Figure 7.A3 (*Continued.*)
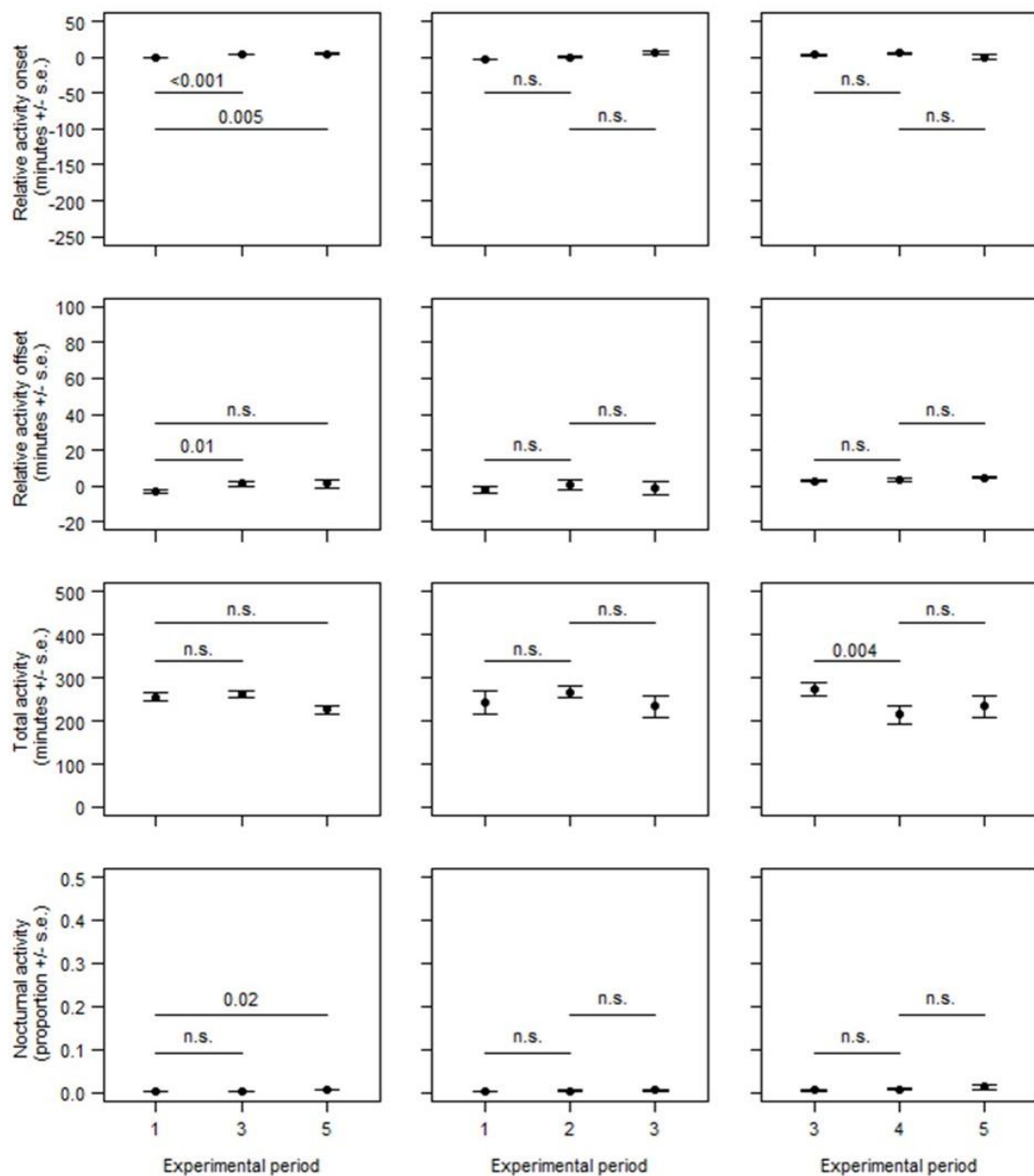
Figure 7.A4 Plots of comparison of activity variables for experiment 2, for dark night periods 1-3-5 all individuals, periods 1-2-3 individuals with dark treatment in period 2, and period 3-4-5 individuals with dark treatment in period 4. Plotted on the same y-axis scales as Figures 7.1 and 7.2. For plotting purpose individual variation was taken out (i.e. data was standardized per individual).

Chapter 8

General discussion

8.1 Main findings in this thesis

The basis of this thesis was formed by the worldwide disappearance of dark nights due to light pollution, the potential large impact of this on ecosystems, of which birds often form an important component, together with the possibilities of reducing this impact by adapting management strategies, such as changing the light spectrum. In this thesis, I set out to investigate the effects of artificial light at night, on the ecology of birds. More specifically, I studied the effects of different light colours and light intensities, to get more insight in the possibilities of mitigation. I focussed on the effects of artificial light at night on several aspects of ecology, amongst which physiology, behaviour, life-history traits and fitness, in common songbirds in the Netherlands. To this end, I have been using three different approaches: 1) I performed a correlational study using long term data from across the Netherlands, relating avian timing of breeding to levels of light pollution; 2) a large-scale field experiment was set up, in which formerly dark, natural habitat is experimentally illuminated and the breeding ecology of cavity-breeding passerines is studied; and 3) in a controlled environment, I studied the effects of night-time light colour and intensity on the physiology and behaviour of captive birds.

In the Netherlands, one of the most urbanized countries with more than 90% of the population living in urban areas (United Nations 2014), city wildlife and urban ecology form an increasingly important part of nature conservation and therefore it is important to measure consequences of urbanization for biodiversity. With the study in **Chapter 2** I aimed to get insight in how timing of avian breeding is influenced by the level of urbanization of an area, for which I used nocturnal light level as a proxy. I used data collected by the citizen science network NESTKAST, from ten common, nest box breeding, bird species. There were no consistent effects of light levels on average first egg laying dates, but for great tit (*Parus major*), blue tit (*Cyanistes caeruleus*) and pied flycatcher (*Ficedula hypoleuca*), we did observe a correlation between lay dates and nocturnal light. In most years the effect of light level was not significant, but when it was, the direction of the relationship was variable; negative in some years and positive in another. The main shortcoming of this study is that it includes few data from the highly urbanized, western part of the Netherlands, with a dense human population and high levels of light pollution. Therefore, in order to truly quantify the impact of urbanisation on wild birds in the Netherlands, data collection first needs to be expanded into more urban areas.

The unique, large scale, experimental set-up of the Light on Nature project (Spoelstra et al. 2015) is designed to assess the effects of three different colours

of street lighting on several species groups. At eight study sites, previously dark, natural habitat is illuminated with white, green or red light, in addition to a dark control. The experimental nature of the set-up allows for testing the effects of nocturnal illumination, independent of other anthropogenic disturbances normally associated with light at night. In this set-up, I measured the effects of light at night on life-history traits and fitness components in two free-living songbird species, the great tit and the pied flycatcher, in two consecutive years. In **Chapter 3** I showed in 2013, but not in 2014, an advancing effect of white and green light on first egg laying date, and an effect of the interaction of treatment and distance to the nearest lamp post on chick mass in great tits, but not in pied flycatchers. In neither species I found an effect of light treatment on breeding densities, clutch size, probability of brood failure, number of fledglings and adult survival. In the same two years, I studied the extra-pair behaviour of the great tit. In **Chapter 4** I determined the parentage of all great tit offspring and tested for an effect of light at night and distance to the light on the occurrence of extra-pair paternity and male reproductive success. In 2014, the proportion of extra-pair young in broods increased with distance to the red and white lamps, but decreased with distance in the dark control. In 2013 however, I found no effects on the proportion of extra-pair young. The total number of offspring sired by a male was unaffected by artificial light at night. Overall, the effects of light at night that have been revealed are, so far, not consistent between years and also not between light colours. In the study in **Chapter 3** the effects show only in 2013, and I argue that this might be due to the climatic conditions of that year; a very cold and late spring which might have caused the artificial light to have a bigger effect than usual. In the study in **Chapter 4**, on the other hand, the effects show only in 2014, and I mainly link this to the higher sample size in that year, probably also partly due to the climatic conditions. Regarding the effect of light colour; timing of breeding was only affected by white and green, whereas extra-pair paternity was affected by all three light colours, but most strongly by white and red. In general, the effects brought to light in these two studies, are alterations in behaviour of birds. Whether birds in illuminated nights are doing better or worse in the long run, compared to birds in dark nights, remains to be seen. So far, I observed no fitness consequences of breeding in lighted areas, in terms of reproductive success of pairs, or reproductive success of males when extra-pair offspring is included, or of adult survival to the next breeding season. Besides the results described in this thesis, I have been closely involved with work by Ouyang et al. (2015), which showed at the same experimental sites that light at night can increase corticosterone levels in the great tit. I attempted to give insight in whether the effects found on birds in the Light on Nature experiment were direct or rather indirect effects of artificial light at night in **Chapter 5**. By deploying male great tits with a light logger measuring light intensity every five minutes over a 24

hour period, I aimed to quantify the nocturnal light exposure of birds in this set-up. I show that the light levels experienced by the male great tits nesting in the direct surroundings of the lamp posts are much lower than the light levels measured there, and that males from pairs breeding close to the lamp posts were not exposed to more light than males from pairs nesting further away. Thus, **Chapter 5** suggests that male great tits avoid exposure to light at night, and that therefore the effects found so far (in **Chapters 3**, **4** and in Ouyang et al. 2015) may be indirect rather than direct. I am aware that the sample size of data from birds with light loggers is quite low, but this kind of work has not been done before and I present the first measurements of night-time light levels on small passerines, in an experimental set-up. More measurements are definitely needed to be conclusive about the findings. If the effect on lay date is indeed indirect, the absence of an effect in red light does make sense; food availability is, next to photoperiod, a very important driver of first egg laying date, and insects, a main food source for great tits during the breeding season, are especially strongly attracted to light with short wavelengths (here the green and white lamps) (van Grunsven et al. 2014). A possible explanation could be that there was more food available under white and green light and birds therefore were able to start breeding earlier.

In the bird facilities at the Netherlands Institute of Ecology (NIOO-KNAW), I studied the effects of night-time light colour and intensity on the physiology and behaviour of hand-raised great tits and blue tits. The main advantage of these kind of 'laboratory' studies is that I could really focus on measuring specific effects, without other variables being confounding, and be sure that birds were actually exposed to the intended light levels. This in contrary to the previously described field studies, where, as in most ecological field experiments, many factors play an unmeasurable role and, additionally, birds can easily avoid light exposure at night. In the experimental study in **Chapter 6**, I demonstrated dose-dependent effects of artificial light at night on birds' daily activity patterns and melatonin levels, which had not been done before. Activity onset was increasingly advanced, and activity offset delayed with higher light intensities. Besides, night-time activity increased and melatonin levels measured at midnight decreased with higher intensities. With two experiments on blue tits, performed using the same experimental set-up, I took these results one step further; in **Chapter 7** I looked at the effects of light colour and light intensity on daily activity patterns. In all colours, but most in red and white light, birds advanced their onset of activity in the morning. The offset of activity was slightly delayed in all light colours. The total activity of birds over a 24 hour period did not change due to light, but part of their daily activity was moved into the night, proportional to the advancement of onset. Because of a significant difference in advancement of onset of activity between green and white

(or red) light, I investigated the intensity-dependent effects of green and white light. The effect of light intensity on activity onset was smaller in green than in white light in the lower range of intensities, but became equal in the highest intensities. Results for offset, total activity and nocturnal activity were consistent with the light colour experiment. These findings show that there are differences in effects of light at night between light colours and that the strength of disturbance of daily activity patterns can, to a moderate degree, be mitigated by changes in characteristics of outdoor lighting. The effects revealed in **Chapters 6** and **7** are different to some extent. Two passerine study species have been used and it could well be possible that these differ in their spectral sensitivity and hence perceive different light colours as different intensities. For blue tits, the wavelengths of maximum sensitivity are known (Hart et al. 2000), for great tits however, they are not. There was also one major, consistent result between the studies. Avian daily rhythms were always, most strongly affected in the morning (onset of daily activity), whereas the effects on offset of daily activity were much more limited. This is a general pattern observed in songbirds and in line with earlier studies on effects of light at night on daily activity (Miller 2006; Dominoni et al. 2014).

8.2 Some notes on the methodology

The study species which I focussed on in my experimental field studies are the great tit and the pied flycatcher, and in my laboratory studies these are the great tit and the blue tit. Where great and blue tits are staying in the Netherlands year-round, and most probably also at the experimental field sites, pied flycatchers are migratory birds that arrive at their breeding grounds shortly before the first eggs are laid, and leave again for Africa after summer. Summed up, or yearly, exposure to light at night does therefore differ a lot between these species, and could very well explain why we only found an effect of artificial light at night on first egg laying dates in great tits and not in pied flycatchers (**Chapter 3**). Exposure to nocturnal illumination might not be long enough to affect timing of egg laying in pied flycatchers. Besides, the short time span between arrival and egg laying leaves little possibility for advancement of laying date for pied flycatchers. Furthermore, great tits and blue tits are common birds in cities, and are also breeding regularly in urban areas. This indicates that effects found, or maybe actually those not found, such as effects on fitness, could originate from adaptation to urban habitats and artificial light at night (Swaddle et al. 2015). Although the field sites used in this study were previously unlit areas, birds with the behavioural plasticity to adapt fast to novel conditions, a common characteristic of urban birds (Evans et al. 2011), may have been able to change their behaviour and / or physiology as such that light at night has no negative consequences for them. On the other hand, if their phenotypic changes were not adaptive (enough), fitness effects

should become visible. Birds that do not show behavioural or physiological plasticity where the novel conditions ask for this, will have a lowered fitness. It could also be that the anthropogenic influences on the environment simply do not need plasticity. If there occurs adaptation on a longer term, it should become visible as a difference between the dark control and the light treatments, and it is very well feasible that, so far, I did not yet measure the specific behavioural or physiological characteristics that were altered.

At the experimental field sites, individual birds are free to choose where they breed, i.e., in which light treatment and at which distance to the lamp posts. Thus, a non-random selection of the population may breed in nest-boxes under light at night. High quality birds may occupy the best places, or the earliest birds may take the most preferred ones. In **Chapter 3** I do show that the breeding density did not differ between light treatments, and that birds that survived from 2013 to 2014 did not move to a particular light colour or away from the illuminated area to the dark control. It is thus not likely that there is a high preference for certain nest boxes. However, e.g., regarding the results in **Chapter 4**, it cannot be excluded that males that were more attractive (and hence are mated to females that are less likely to engage in extra-pair copulations) settled in more illuminated territories, and that this fact causes the findings. Ultimately, it would be best if I could attribute a light treatment to all breeding pairs and 'place' them in a specific nest box, such that the allocation of birds to treatments would be really random. In the current set-up this is not possible (but see ideas for further research at the end of this chapter).

Two main advantages of the laboratory studies in **Chapters 6** and **7** were that I was able to keep many factors constant between light treatments, and that I could precisely dose the light exposure of the individual birds, which are both major problems in the field experiment. However, a main shortcoming of these kind of studies is that they are short term, a few months at most. This, together with the fact that birds are fed *ad libitum*, makes it difficult to measure any fitness consequences. These studies are perfect for measuring direct effects on behaviour and on physiology, but it is hard to relate these effects to fitness effects in the wild. Still, to answer questions about effects of artificial light at night at a mechanistic level, studies such as those in **Chapters 6** and **7** are highly valuable. Besides, longer term studies in a controlled environment where birds can be kept in breeding pairs, open up the possibility to study the direct effects of light at night on life-history traits (see also future research).

8.3 Effects of light at night at different organisational levels

The effects of light at night can take place at different organisational levels (Figure 1.3 in **Chapter 1**). Firstly, effects on the physiology, behaviour and life-history traits of an individual, either directly caused by light at night, or indirectly, via effects of light at night on the environment, which influences the individual. There are plenty of examples of these kind of effects. In this thesis, I have shown direct effects of nocturnal illumination on daily activity patterns (**Chapters 6** and **7**), on plasma melatonin levels (**Chapter 6**) and on light avoidance behaviour during roosting (**Chapter 5**). Effects on timing of breeding (**Chapters 2** and **3**) and on extra-pair behaviour (**Chapter 4**) could be direct, or via the environment which is affected by light at night as well. Other physiological effects have been shown by Ouyang et al. (2015); corticosterone concentrations were increased under light at night at the Light on Nature study set-up. These findings add to the effects on behaviour of birds that were already known, such as the alteration of dawn and dusk singing (Da Silva et al. 2015), the extension of foraging into the night (Russ et al. 2015), and the advancement of lay date, an important life-history trait, that was found in blue tits (Kempenaers et al. 2010). The strong effects of light at night on the daily activity patterns of great tits and blue tits (**Chapters 6** and **7**) and those earlier shown in blackbirds (Dominoni et al. 2013b), raise the question whether these modifications are reflected by properties of the endogenous circadian clock. A comparison of forest and city blackbirds has related variation in daily timing to the properties of the underlying circadian clock, which showed to be faster but also more disrupted in urban birds (Dominoni et al. 2013c). As a follow-up of work in this thesis, Spoelstra et al. set out to measure the endogenous circadian rhythm in birds that were exposed to different levels of white light at night. Preliminary results show that free running rhythms were not affected in great tits, despite the large effects on daily activity pattern (Spoelstra et al. in prep.). Also linking to the disturbance of activity patterns and especially to the alterations of nocturnal activity that were found in the laboratory studies, together with Jenny Ouyang we equipped great tits in our Light on Nature experimental set-up with radio telemetry tags, in order to measure their activity when roosting in illuminated areas. Birds under white nocturnal illumination had significantly higher night-time activity levels than birds in the other light colours, and than those in the dark (Ouyang et al. in prep.). These results indicate that light at night disturbs sleep in wild birds (see also Raap et al. 2015).

Secondly, effects on an individual's fitness may arise directly, for example mortality of nocturnally migrating birds through attraction to light from lighthouses (Jones and Francis 2003), or indirectly, via previously mentioned effects on physiology, behaviour or life-history traits. The latter are the type of effects on

fitness that could be observed in birds at the Light on Nature experimental set-up, however in **Chapters 3** and **4** I show that, apart from fledgling mass which seems to be affected but only in one out of two years, there are no apparent effects on fitness of individual birds. There are very few examples of non-direct effects of light at night on fitness in earlier studies. One of them is by Kempenaers et al. (2010), who did show that male blue tits breeding in illuminated territories were more successful in obtaining extra-pair mates. Effects of light at night on fitness may however be very latent, and long-term research is the only way to investigate these. Besides, lamps are actually never installed for short terms, so it does make sense to ultimately focus on studying the long-term effects.

Then, thirdly, the combined direct and indirect effects on the fitness of individuals may cause effects on the population level. If individual birds do better or worse in illuminated nights, this could, but does not necessarily have to, show at population numbers (see for example Reed et al. 2013). In this thesis, I do not include measurements of bird population numbers, although there have been measurements done over the past five years in the Light on Nature set-up. Spoelstra et al. (2015) published the first results on bird population data. All bird species were surveyed by professional field ornithologists from the Dutch Centre for Field Ornithology (Sovon), who visited each site eight times during each breeding season. Whether birds were considered breeding was determined on the basis of a combination of species specific behaviour, presence during a certain time window and presence during consecutive visits. Light treatment had a clear effect on the year-to-year change in presence of individual species, and the change in numbers was most positive at the illuminated transects. It is not yet clear what has caused this. If these trends observed during the first two years of the monitoring continue, this would imply that densities of birds will increase at the illuminated sites. Another set of data that has been collected at the Light on Nature field sites has not yet been published. Birds were caught and ringed with a uniquely numbered aluminium ring during six mornings per breeding season with 50 meter mist nets at each transect. This was done by volunteers and coordinated by the Dutch Centre for Avian Migration and Demography (Vogeltrekstation), in order to measure the effects of light at night on recruitment and survival of birds.

Finally, when looking at Figure 1.3 for populations of several different species, which may be part of a food web, one can imagine that each species influences the populations of the others and that this may lead to cascading effects of artificial light at night. Bennie et al. (2015) give a nice example of these kind of effects in a grassland ecosystem. In the Light on Nature set-up, many different species groups are being monitored, all at the same locations. This is unique in

the Netherlands, and opens up the possibility to study the interactive and cascading effects on the whole forest-edge ecosystem. For example, great tits are strongly dependent on the peak of caterpillar biomass for feeding their young during the breeding season. Changes in temperature can affect the phenology of great tits and their prey species in a different way, which then could cause a mismatch in timing (Visser et al. 2006). Artificial light at night can potentially have the same cascading effect. At the Light on Nature field sites, flying insects and caterpillar biomass have been measured consistently over respectively one and two breeding seasons. Together with the standard monitoring of moths at these sites, it will be possible to link abundance and timing of insects to the breeding biology of birds.

8.4 Implications for policy and conservation

Some of the findings presented in this thesis can be directly translated into advice for policy and conservation, others first need further investigation. For example, in **Chapter 3** I stated that the non-consistent effects that I found, indicate the need for long term studies. And I conclude that, if the magnitude and direction of possible effects depend on the spectral composition of light at night, this could open up the possibility to mitigate specific ecological consequences with the use of coloured nocturnal illumination. The disruption in daily rhythms, presented in **Chapter 6**, is especially relevant in urbanised areas, where light levels are increased over large areas, and can have potentially negative effects for an animal's fitness. However to show this, future studies should first focus on the effects of different intensities of light at night in the wild.

The dose-response relationship of change in activity pattern versus light intensity which I have shown in both **Chapters 6** and **7**, indicates that decreasing light intensity could be a measure to reduce the effects of artificial light at night. Also, I have shown that there are differences in effects on avian daily rhythms between light colours (**Chapter 7**) and that green light at low light intensities disturbs daily activity patterns less. This means that the strength of the disturbance of behaviour of wild birds can be mitigated by changes in the characteristics of outdoor lighting, which opens up possibilities for conservationists and landscape planners to use this in their daily practice.

The advices so far are based on findings for just three species. These may be extrapolated to other bird species, although with some caution, but certainly not directly to other species groups. At least not without further research on the effects in those groups. Since the effects of light at night of different spectral compositions do vary wildly between species groups (Musters et al. 2009;

Spoelstra et al. 2015), the challenge is to in come up with advices for an area rather than for just one species or species group. If natural areas are appointed as habitat for specific species, these species should then maybe be the focus when it comes to advice on lighting. However for areas where this is not the case, it might be more difficult when making decisions on outdoor lighting.

A present day example from the Netherlands comprises the wide application of green LED lamps in outdoor lighting. Poot et al. (2008) have shown that nocturnally migrating birds got less disturbed by brightly illuminated oil rigs amid a dark sea when these were illuminated with green, instead of white light. Removing long wavelengths from the light spectrum makes migrating birds much less attracted and disoriented by illuminated spots (Wiltschko et al. 1993; Wiltschko and Wiltschko 1995). Although it is highly relevant to apply these findings at sea, at for example oil platforms, and at coastal roads, coastal parking lots etc., in terrestrial ecosystems, i.e. the rest of the Netherlands, this is not necessarily the case. The positive effect of green light found at sea, should thus not be generalized that easily.

8.5 Recommendations for future research

For each of the three methodological approaches used in this thesis, I will give some recommendations for future research. Starting with the correlational field study (**Chapter 2**), I strongly recommend expanding data collection on timing of avian breeding and avian fitness into more urban areas, and to include more study areas in highly lighted parts of the Netherlands in the NESTKAST project, in order to quantify the impact of urbanization on wild bird species. The infrastructure to do this is actually already there, in the form of the 'Nestkaart Light' project of Sovon. This project gives citizen scientist the opportunity to enter their nest box breeding bird data online and with little effort. The only step still to be taken is to make more people, and especially those living in urban areas, enthusiastic for this kind of bird research. At the same time the opportunity should be taken to create awareness of the light pollution problems and to show people the aesthetic value of dark nights.

The Light on Nature experimental field set-up has been running now for five years and the first results have been published. There are some interesting findings, for example that there are fewer extra-pair offspring in illuminated broods, which thus shows that light at night disrupts sexual selection processes (**Chapter 4**). Evidence of a variety of effects of light at night on birds is accumulating, but still many questions remain to be answered. What are the larger scale consequences of light at night? And, are bird populations affected in areas with night-time illumination?

Over a longer time period, long-term effects are expected to become visible, not only for birds, but for all species groups. The Light on Nature experimental design creates the opportunity to answer these kind of questions. Therefore I recommend to continue to record data on nest box breeding birds as well as to continue the monitoring of breeding birds and the capture and recapture by mist netting. Especially the combination of data collection on birds using three different methods at the same locations studying the effects of artificial light at night is highly interesting. The amount of data that was collected over the previous five years can give new insights when the data from the different methods will be combined. For example by using an 'integrated population model', which integrates all available demographic data, such as capture-recapture data, reproductive data and survey data, into one model (Schaub and Abadi 2011). Advantages of these kind of models are that otherwise inestimable demographic parameters can be estimated, and that all demographic processes are included in one model.

To overcome the problem that is faced in the Light on Nature set-up with non-random allocation of birds to treatments (**Chapter 3**), it is necessary to perform additional field experiments. A possibility would be a set-up where a light treatment can be attributed to a breeding pair in a specific nest box. The birds would choose a 'normal', dark territory and after they have settled there, the randomly allocated treatment (green, white or red light, or dark control) would start. Their whole territory should be illuminated, for example by a single lamp post next to the tree with the nest box, to enable also indirect effects of the light to show. This way the effects of light at night on timing of breeding and breeding success can be assessed, without the confounding effects of settlement of birds with specific characteristics. The down side of this approach would be that the period that birds are exposed to light at night will be rather short, when the treatment starts only after settlement, and effects may actually arise already in the period before. Another way of studying the direct effects of light at night on seasonal timing is doing this in captivity. In collaboration with Kees van Oers, we provided breeding pairs of great tits in outdoor aviaries with white light at night, of intensity comparable to that measured in the experimental field set-up. First egg laying dates were monitored and compared to those of breeding pairs without light at night. Preliminary results of data from two consecutive years show that the timing of breeding in this experiment was not affected by light at night (de Jong et al. in prep.). This indicates again that, confirming the findings in **Chapter 5**, the effects found on timing of egg laying in the wild (**Chapter 3**) are probably indirect effects rather than direct effects of nocturnal illumination.

In **Chapter 6** I show a strong effect of night light intensity on melatonin levels at midnight; the concentrations decreased with increasing light levels. For the lower light intensity treatments, levels were higher under illuminated nights compared to dark nights. I hypothesized that (see Appendix of **Chapter 6**) the daily rhythm of melatonin secretion shifts as an effect of artificial light at night, following the advancement in onset of activity. The peak in melatonin level which normally, during dark nights, would be reached after midnight, might now be advanced and reached around midnight (in low light levels, leading to high melatonin) or even much earlier in the evening (in high light levels, leading to lower melatonin than under normal, dark conditions). This is a post-hoc explanation of my findings which should be tested further, for instance by sampling birds more times per night, in the future. As a follow-up of the experiment presented in **Chapter 6**, in close collaboration with Davide Dominoni and Barbara Helm, we examined the regulation of clock genes and of glucocorticoid (stress) receptor genes, and metabolomic profiles under light at night, in order to further assess the mechanisms involved in the effects of light on daily rhythms (Dominoni et al. in prep.). Laboratory experiments are a valuable tool in investigating the effects of light at night on birds, given that they contain a strong link to field experiments and that application of the results should be possible. For example by choosing the use of light spectra and intensities that are relevant for outdoor lighting (**Chapter 7**). Too often, laboratory studies on the effects of light at night use unnaturally high levels of light or they compare light levels in units other than lux, which makes results hard to interpret in terms of outdoor lighting options. A recent review by Dominoni et al. (2016) also concludes that merging the mechanistic approach of chronobiologists with ecological field studies measuring e.g. health and longevity of wild animals, is the way forward in discovering the proximate mechanisms as well as the ultimate consequences of artificial light at night.

With this thesis, I started uncovering the effects of artificial light at night on avian ecology. The field that is studying ecological consequences of light pollution has evolved rapidly over the last couple of years. Compared to when I started my PhD, about four years ago, we have come to know a lot more regarding the biological impacts of light at night. Still, there is much more to be discovered. In my thesis, I have presented some novel findings on birds in illuminated nights and I have given some recommendations for future research. To conclude, I hope that you, as a reader of this thesis, will realize that dark nights have almost disappeared in our urbanised, Western world, and that, when living there, you belong to the lucky few if you are able to see the Milky Way or 'just' thousands of stars – even when that is only some nights a year.

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English and Dutch summaries

Natural night-time darkness has disappeared across large parts of the world as a result of light pollution, the alteration of light levels in the outdoor environment due to artificial light sources. This increase in sky brightness not only obscures our perception of the starry sky; it can also have severe effects on human health as well as detrimental impacts on nature. Many animals are attracted to light at night and thereby suffer direct fitness losses, but more subtle effects can also occur. One such effect is the timing of daily and seasonal activities. As organisms have evolved under a natural light-dark cycle, which is the main driver for circannual and circadian rhythms, these activities can be disturbed by anthropogenic light at night.

One species group that is potentially greatly affected by nocturnal illumination is birds, as species in this group have excellent vision and also possess light sensitive tissue in their brain. Artificial light at night can thus affect many aspects of a bird's life. Timing of dawn and dusk singing, foraging behaviour, and sleep pattern are for example known to be altered by the presence of street lights. Yet, so far, experimental and especially long term field studies investigating the effects of nocturnal illumination on birds are lacking.

Amongst the options to reduce the effects of night-time light pollution on ecosystems is the adaption of the light spectrum. The LED lamps that are used more and more in outdoor lighting have large economic advantages, and their colour composition can be custom-designed. This could potentially mitigate the impact of light on flora and fauna by using a specific light colour that has minimal effects on biological processes. However, little is known about the effects of different colours of light on birds.

The aim of this thesis was to investigate the effects of artificial light at night on the ecology of birds. More specifically, the effects of different light colours and light intensities are studied, to provide insight into the possibilities of mitigation. The focus was on investigating the effects of artificial light at night on several aspects of avian ecology, including effects on physiology, behaviour, life-history traits, and fitness, by studying common songbirds in the Netherlands. To this end, three different approaches were used.

Firstly, avian timing of breeding was related to levels of light pollution, in a correlational study using long-term data from across the Netherlands. Nocturnal light levels were used as a proxy for the level of urbanisation of an area. Data from ten common, nest box breeding bird species, collected by a citizen science network, were used. For great tits (*Parus major*), blue tits (*Cyanistes caeruleus*) and pied flycatchers (*Ficedula hypoleuca*), correlations were found between light

levels and first egg laying dates. However, these correlations were not apparent in all years, and were negative in some years and positive in others. A possible reason for this inconsistency is that this study included few data from the highly urbanized areas of the Netherlands. In order to truly quantify the impact of urbanisation on wild birds, data collection needs to be expanded to include such areas.

Secondly, in order to study the breeding ecology of cavity-breeding passerines, a large-scale field experiment was set up in which formerly dark, natural habitat was experimentally illuminated with white, green or red LED light, in addition to a dark control treatment. The experimental nature of the set-up allowed the effects of nocturnal illumination to be tested independently of other anthropogenic disturbances that are normally associated with light at night. The effects of light at night on life-history traits and fitness components in two free-living songbird species, the great tit and the pied flycatcher, were measured in two consecutive years. In 2013, but not in 2014, white and green light advanced the first egg laying date of great tits. Pied flycatchers were unaffected by the light at night. In the same two years, the extra-pair behaviour of the great tit was studied. In 2014, the proportion of extra-pair young in broods increased with distance to the red and white lamps. In 2013, light had no effect. The discrepancies between years in both studies were probably linked to the very different climatic conditions in both years. The effects that were shown in these studies are with regard to the behaviour of birds; so far in our experimental study, no fitness consequences of breeding in lighted areas have been observed, in terms of the reproductive success of pairs, the reproductive success of males when extra-pair offspring is included, or of adult survival to the next breeding season. To study whether the behavioural effects found so far at this experimental set-up were due to the direct or the indirect effects of artificial light at night, male great tits were deployed with light loggers to measure their light exposure over a 24 hour period. Males from pairs breeding close to the lamp posts were not exposed to more light than males from pairs nesting further away. This suggests that male great tits avoid exposure to light at night and thus that the effects of artificial light on behaviour found so far might be indirect rather than direct.

Finally, the effects of night-time light colour and intensity on the physiology and behaviour of captive birds were studied, in a controlled laboratory environment. In contrast to the field studies, in this set-up birds were exposed to artificial light levels with no possibility of escaping to darker places. Dose-dependent effects of artificial light at night on birds' daily activity patterns and melatonin levels were demonstrated in great tits. Higher light intensities advanced activity onset and delayed activity offset. Night-time activity increased and melatonin levels

(measured at midnight) decreased at higher light intensities. In two other experiments, the effects of light colour and light intensity on the daily activity patterns of blue tits were studied. In all colours, and most of all in red and white light, birds advanced their onset of activity in the morning. The effect of light intensity on activity onset was smaller in green than in white light in the lower range of intensities, but became equal at the highest intensities. These studies show that different light colours have different effects on activity patterns, and that disturbance to daily activity patterns can be partially mitigated by changes in the characteristics of outdoor lighting.

Some of the findings presented in this thesis can be directly translated into advice for policy and conservation; others first need further investigation. Furthermore, all are based on studies of a few bird species. Since the effects of light at night of different spectral compositions do vary widely between species groups, the challenge is going to be in coming up with advice on outdoor lighting for areas with many different species, rather than for just one species or species group. This thesis has begun to uncover the effects of artificial light at night on avian ecology; some novel findings on birds in illuminated nights have been presented and recommendations for future research have been made. In recent years, much has been revealed regarding the biological impacts of anthropogenic nocturnal illumination. Yet, there is much still unknown. The experimental field set-up described here forms an excellent and valuable tool to continue to study the effects of ever-increasing night-time light levels on ecosystems.

Als gevolg van lichtvervuiling zijn donkere nachten in grote delen van de wereld verdwenen. De verhoogde hemelhelderheid zorgt er niet alleen voor dat we de sterrenhemel niet goed meer kunnen zien, ook onze gezondheid ondervindt er schadelijke effecten van, en de natuur wordt erdoor verstoord. Veel dieren worden aangetrokken door nachtelijk licht en ervaren daardoor zeer directe, negatieve en soms dodelijke effecten. Ook meer subtiele effecten komen voor. Eén daarvan is de timing van dagelijkse en seizoensgerelateerde activiteiten. Omdat organismen geëvolueerd zijn onder een natuurlijke cyclus van licht en donker, welke de voornaamste aandrijver is van jaarlijkse en dagelijkse rytmen, kunnen deze activiteiten verstoord worden door nachtelijk kunstlicht.

Vogels zijn een soortgroep die potentieel grote invloed kan ondervinden van nachtelijk licht, omdat de soorten in deze groep zeer goed zichtvermogen hebben en ook over lichtgevoelige delen in hun hersenen beschikken. Nachtelijk kunstlicht kan vele aspecten van het leven van een vogel beïnvloeden. De timing van zang rond zonsopgang en zonsondergang, foerageergedrag en slaappatronen staan er bijvoorbeeld om bekend veranderd te worden in de nabijheid van straatverlichting. Tot nu toe missen er echter experimentele, en in het bijzonder lange termijn veldstudies die de effecten van nachtelijke verlichting op vogels onderzoeken.

Eén van de mogelijkheden tot het verminderen van de effecten van lichtvervuiling op ecosystemen is aanpassing van het lichtspectrum, ofwel de kleur van het licht. De LED lampen die tegenwoordig steeds meer in buitenverlichting gebruikt worden hebben grote economische voordelen, bovendien kan hun kleur naar wens worden aangepast. Door gebruik te maken van een specifieke lichtkleur die minimale effecten heeft op biologische processen kan mogelijk de impact van licht op flora en fauna verminderd worden. Er is echter nog maar weinig bekend over de effecten van verschillende kleuren licht op vogels.

Het doel van dit proefschrift was het onderzoeken van de effecten van nachtelijk kunstlicht op de ecologie van vogels. Specifieker; de effecten van verschillende kleuren licht en lichtintensiteiten zijn bestudeerd, om inzicht te verkrijgen in de mogelijkheden tot mitigatie. De focus lag op het onderzoeken van de effecten van licht op een aantal aspecten van de ecologie van vogels, waaronder fysiologie, gedrag, life-history kenmerken en fitness, door algemene zangvogels in Nederland te bestuderen. Hiertoe zijn drie verschillende methoden gebruikt.

Ten eerste is de timing van broeden van vogels gerelateerd aan het niveau van lichtvervuiling, door middel van een correlationele studie waarin gebruik gemaakt is van een lange termijn dataset uit heel Nederland. Nachtelijke lichtniveau's zijn

een schatting voor de mate van urbanisatie van een gebied. Data van tien algemene nestkastbroeders zijn onderzocht, afkomstig van een citizen science netwerk. Voor koolmezen (*Parus major*), pimpelmezen (*Cyanistes caeruleus*) en bonte vliegenvangers (*Ficedula hypoleuca*) zijn er correlaties gevonden tussen lichtniveau's en eerste eilegdata. Echter deze correlaties waren niet aanwezig in alle jaren en waren negatief in sommige en positief in andere jaren. Een mogelijke reden voor deze inconsistentie in de resultaten is dat deze studie relatief weinig data van de meest urbane gebieden in Nederland bevatte. Om daadwerkelijk de impact van urbanisatie op broedgedrag van wilde vogels te kwantificeren is het nodig de verzameling van data naar meer urbane gebieden uit te breiden.

Ten tweede is een grootschalig veldexperiment opgezet om de broedecologie van holenbroedende zangvogels te bestuderen. Hierin is voorheen donkere, natuurlijke habitat experimenteel verlicht met witte, groene of rode LED verlichting, naast een donkere controle behandeling. Het experimentele karakter van deze opzet maakt het mogelijk de effecten van nachtelijke verlichting te testen, onafhankelijk van andere door mensen veroorzaakte verstoringen welke normaal gesproken gepaard gaan met nachtelijk licht. De effecten van nachtelijk licht op life-history kenmerken en componenten van fitness zijn gemeten in twee opeenvolgende jaren, in de zangvogels koolmees en bonte vliegenvanger. In 2013, maar niet in 2014, vervroegden koolmezen hun eerste eilegdatum door de witte en groene verlichting. Bonte vliegenvangers ondervonden geen effect van het licht. In dezelfde twee jaren is het buitenechtelijke paringsgedrag van koolmezen bestudeerd. In 2014 was het aandeel buitenechtelijke jongen in broedsels groter naarmate de afstand tot de rode en witte lampen toenam. In 2013 was er geen effect van het licht. De onregelmatigheden tussen de jaren in deze beide studies zijn waarschijnlijk gelinkt aan de grote klimatologische verschillen tussen deze jaren. De effecten die zich geopenbaard hebben zijn effecten op het gedrag van vogels; tot dusver zijn er in deze experimentele studie geen consequenties voor fitness gevonden van broeden in verlichte gebieden. Niet in het reproductieve succes van paartjes, niet in het reproductieve succes van mannetjes, ook wanneer buitenechtelijke jongen meegeteld worden, en ook niet in de overleving van volwassen vogels naar het volgende broedseizoen. Om te bestuderen of de effecten op gedrag die gevonden zijn in deze onderzoeksopzet te wijten zijn aan directe of indirecte effecten van nachtelijk kunstlicht, zijn mannelijke koolmezen uitgerust met een lichtlogger om hun blootstelling aan licht te meten gedurende een periode van 24 uur. Mannen van paartjes die dichtbij de lantaarnpalen broeden zijn 's nachts niet aan meer licht blootgesteld dan mannen van paartjes die verder bij het licht vandaan broeden. Dit suggereert dat mannelijke koolmezen de blootstelling aan nachtelijk licht vermijden en dus ook

dat de effecten van kunstlicht op gedrag die tot dusver gevonden zijn eerder indirect dan direct zullen zijn.

Als laatste zijn de effecten van de kleur en de intensiteit van nachtelijk licht op fysiologie en gedrag van vogels in een gecontroleerde omgeving bestudeerd. In tegenstelling tot in veldstudies, kunnen in deze onderzoeksopzet de vogels blootgesteld worden aan nachtelijk licht zonder dat zij kunnen ontsnappen naar donkerdere plekken. Dosis-afhankelijke effecten van nachtelijk kunstlicht op de dagelijkse activiteitspatronen en de melatonine concentratie zijn gedemonstreerd bij koolmezen. Hogere lichtintensiteiten vervroegden de aanvang van activiteit in de ochtend en verlengden de activiteit in de avond. Nachtelijke activiteit werd hoger en de nachtelijke melatonine concentratie werd lager bij hogere lichtintensiteiten. In twee andere experimenten werden de effecten van lichtkleur en -intensiteit op de dagelijkse activiteitspatronen van pimpelmezen onderzocht. Vogels vervroegden de aanvang van activiteit in de ochtend in alle kleuren, en het meest in rood en wit licht. Het effect van lichtintensiteit op aanvang van activiteit was kleiner in groen dan in wit licht in de lagere intensiteiten, maar werd gelijk voor beide kleuren in de hogere intensiteiten. Deze studies laten zien dat verschillende lichtkleuren verschillende effecten kunnen hebben op activiteitspatronen en dat de verstoring van dagelijkse activiteit gedeeltelijk gemitigeerd zou kunnen worden door bepaalde eigenschappen van buitenverlichting aan te passen.

Sommige van de bevindingen die gepresenteerd zijn in dit proefschrift kunnen direct vertaald worden naar advies voor natuurbeleid en –bescherming, andere zullen eerst nader onderzocht moeten worden. De uitdaging voor de toekomst zal liggen in het vormen van advies over buitenverlichting voor gebieden met veel verschillende soorten, in plaats van voor één soort of soortgroep, omdat de effecten van nachtelijk licht van verschillende spectrale samenstelling zeer uiteenlopen tussen soortgroepen. Dit proefschrift heeft een begin gemaakt met het blootleggen van de effecten van nachtelijk kunstlicht op de ecologie van vogels. Een aantal nieuwe bevindingen over vogels in door de mens verlichte nachten zijn gepresenteerd en aanbevelingen voor toekomstig onderzoek zijn gedaan. In de laatste jaren is er veel ontdekt over de biologische impact van lichtvervuiling. Echter er is ook nog steeds veel onbekend. De experimentele onderzoeksopzet die hier is beschreven vormt een excellente en waardevolle methode om de studie naar de effecten van de nog steeds toenemende nachtelijke lichtniveau's op ecosystemen voort te zetten.

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A personal message to all of you, who have made doing this PhD the wonderful experience that it was. The journey of life now continues, but I will look back at my time at the NIOO with many beautiful memories.
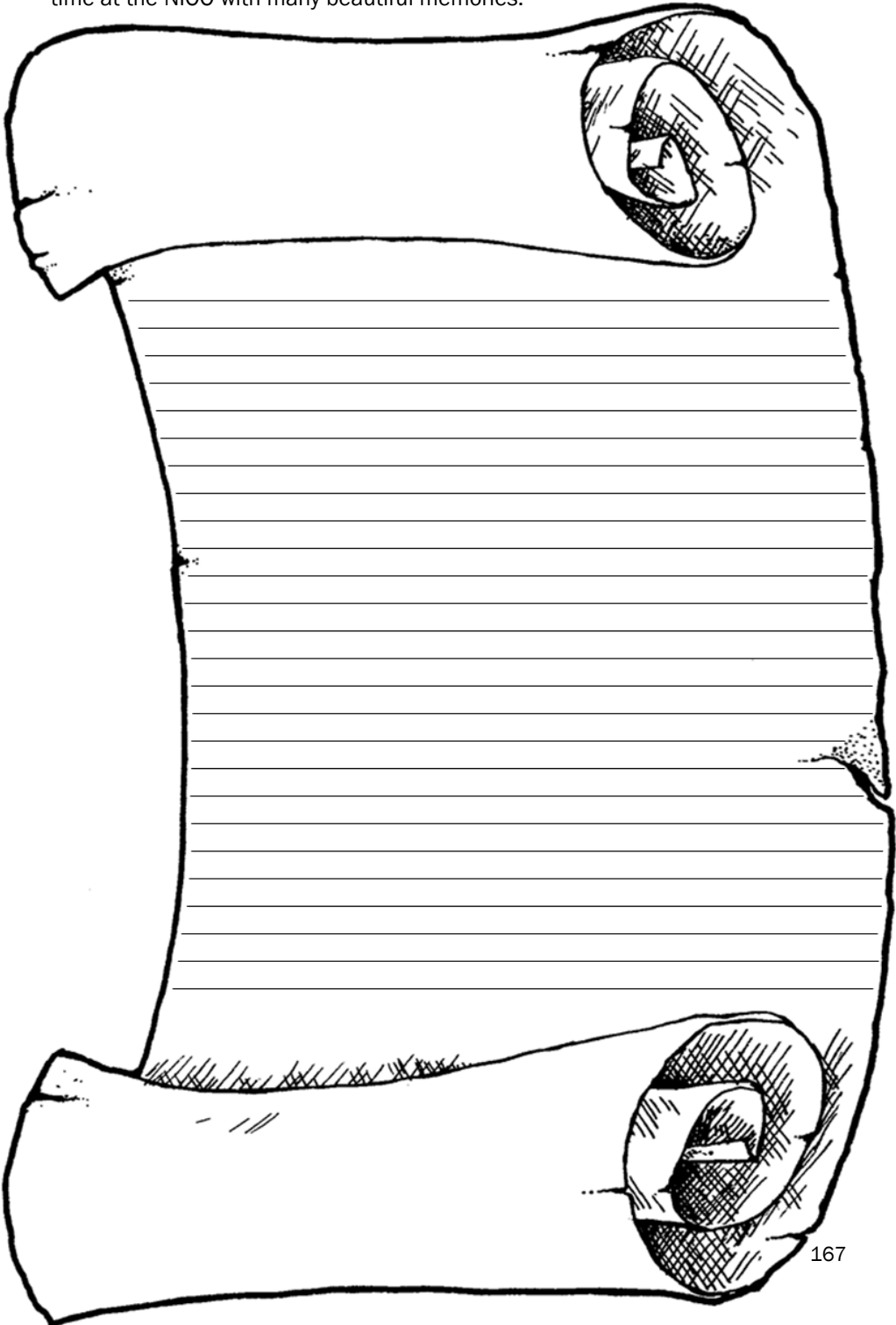
About the author

Maaike de Jong was born on 18 February 1985 in Rotterdam. She grew up in Schoonhoven, where she started exploring the natural world around her already at a young age. After graduating from Het Schoonhovens College in 2003, she moved to Wageningen to study biology. She specialised in ecology and performed several research projects in vegetation ecology and animal ecology, for which she carried out field work in the Peloponnesos and Arctic Sweden. She obtained her bachelor's degree in Biology and in 2010 her master's degree in Forest and Nature Conservation. During her master's, Maaike also followed some didactic courses and did an internship as biology teacher at a secondary school. She started her professional career with Stichting Veldwerk Nederland, educating primary school children about nature and the environment. She was appointed as a lecturer in the bachelor programs Forest & Nature Conservation and Landscape & Environment Management at the University of Applied Sciences Inholland in Delft. In 2012, Maaike started as a PhD candidate at the Netherlands Institute of Ecology (NIOO-KNAW), under the supervision of Marcel Visser and Kamiel Spoelstra. Within the 'Light on Nature' project, which is a collaboration with Wageningen University, she studied the effects of artificial light at night on avian ecology, and became excited about science communication. Her work resulted in this thesis. In the summer of 2016, Maaike will move to Malawi, where she plans to continue doing ecological research, combined with nature conservation and community development, in Ntchisi Forest.

Peer reviewed publications

de Jong M, Jeninga L, Ouyang JQ, van Oers K, Spoelstra K, Visser ME (2016) Dose-dependent responses of avian daily rhythms to artificial light at night. Physiology & Behavior 155:172-179.

de Jong M, Ouyang JQ, Da Silva A, van Grunsven RHA, Kempenaers B, Visser ME, Spoelstra K (2015) Effects of nocturnal illumination on life-history decisions and fitness in two wild songbird species. Philosophical Transactions of The Royal Society B Biological Sciences 370:20140128.

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de Jong M, Ouyang JQ, van Grunsven RHA, Visser ME, Spoelstra K. (2016) Do wild great tits avoid exposure to light at night? PLOS ONE *In press.*

Submitted for publication

de Jong M, van den Eertwegh L, Beskers RE, de Vries PP, Spoelstra K, Visser ME. Timing of avian breeding in an urbanized world. *In revision.*

de Jong M, Lamers KP, Eugster M, Ouyang JQ, Da Silva A, Mateman CA, van Grunsven RHA, Visser ME, Spoelstra K. Cheaters like it dark: female great tits breeding in illuminated areas are more faithful. *Submitted.*

de Jong M, Caro SP, Gienapp P, Spoelstra K, Visser ME. Early birds by light at night: effects of light colour and intensity on daily activity patterns in blue tits. *Submitted.*

Publications in preparation

Ouyang JQ, **de Jong M**, van Grunsven RHA, Matson KD, Haussmann MF, Meerlo P, Visser ME, Spoelstra K. Restless roosts – white light at night affects behavior, sleep and physiology in a free-living songbird.

Da Silva A, **de Jong M**, van Grunsven RHA, Visser ME, Kempenaers B, Spoelstra K. Experimental illumination of a forest: effects on the onset of the dawn chorus in songbirds.

de Jong M, van Bruggen LCG, Lange CYMJG, Spoelstra K, Visser ME. Feeding pattern, nestling diet and begging behaviour of great tits in illuminated nights.

Affiliations of co-authors of thesis chapters

Ronald E. Beskers
NESTKAST, Naarderweg 44, 1261 BV Blaricum, The Netherlands

Samuel P. Caro – Laura van den Eertwegh – Mark Eugster – Phillip Gienapp –
Lizanne Jeninga – Koosje P. Lamers – A. Christa Mateman – Kees van Oers –
Jenny Q. Ouyang – Kamiel Spoelstra – Marcel E. Visser – Peter P. de Vries
*Department of Animal Ecology, Netherlands Institute of Ecology (NIOO-KNAW),
PO Box 50, 6700 AB Wageningen, The Netherlands*

Samuel P. Caro
*CNRS, Centre d'Ecologie Fonctionnelle et Evolutive (UMR 5175), 1919 route de
Mende, 34293 Montpellier Cedex 5, France*

Arnaud Da Silva – Bart Kempenaers
*Department of Behavioural Ecology and Evolutionary Genetics, Max Planck
Institute for Ornithology, Eberhard-Gwinner-Strasse, 82319 Seewiesen,
Germany*

Roy H.A. van Grunsven
*Plant Ecology and Nature Conservation Group, Wageningen University, PO Box
47, 6700 AA Wageningen, The Netherlands
&
Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm
310, 12587 Berlin, Germany*

Jenny Q. Ouyang
*Department of Biology, University of Nevada, Reno, 1664 N. Virginia Street,
Reno, NV 89557, USA*

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities).
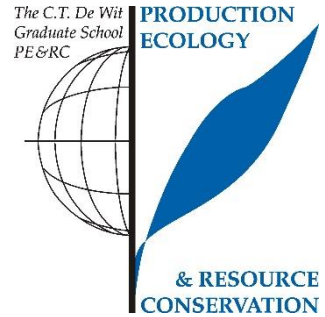
Review of literature (4.5 ECTS)
- Ecology of birds in illuminated nights

Post-graduate courses (6.2 ECTS)
- Laboratory animal science; KNAW (2012)
- Meta-analysis: theory & tips; NVG (2012)
- Life history theory; RSEE (2013)

Laboratory training and working visits (1.8 ECTS)
- Effects of anthropogenic disturbances on behaviour of birds; Max Planck Institute for Ornithology, Radolfzell (2012)
- Artificial light affecting onset of dawn song and extra-pair behaviour; Max Planck Institute for Ornithology, Seewiesen (2014)

Invited review of (unpublished) journal manuscript (1 ECTS)
- Animal Behaviour: Effects of light at night on birds using garden feeding stations (2014)

Deficiency, refresh, brush-up courses (3 ECTS)
- Animal physiology; Utrecht University (2012)
- Functional anatomy; Utrecht University (20120

Competence strengthening / skills courses (1.2 ECTS)
- Presenting yourself & writing proposals; STW (2013)
- Scientific misconduct: the thin line; NVG (2013)
- Organization and time planning & communication in project management; STW (2014)
- Speed reading & communication with media & PhD skills outside academia & writing proposals; WGS (2015)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.1 ECTS)
- PE&RC First years weekend (2012)
- PE&RC Day (2014-2015)
- PE&RC Last years weekend (2015)

Discussion groups / local seminars / other scientific meetings (9 ECTS)
- Light on Nature symposium; organisation (2012-2014)
- R Users group meetings (2012-2015)
- WEES Seminars (2012-2015)
- Netherlands Annual Ecology Meeting (2012-2015)
- Nederlandse Vereniging voor Gedragsbiologie (2012-2015)
- KNAW PhD Students on Science (2015)

International symposia, workshops and conferences (8.8 ECTS)
- INTECOL / BES Annual General Meeting; London (2013)
- 1st International Conference on Artificial Light at Night; Berlin (2013)
- 15th International Behavioral Ecology Congress; New York (2014)
- 2nd International Conference on Artificial Light at Night; Leicester (2014)

Supervision of MSc students (9 ECTS)
- The effects of artificial night light and light colour on extra-pair behaviour in wild great tits
- Does artificial light influence timing of egg laying, clutch size and egg volume of great tits?
- Red light and faithfulness: unwilling females or overprotective males

Colophon